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# RATE AND EXTENT OF DEVELOPMENT OF NEOTENIC REPRODUCTIVES IN GROUPS OF NYMPHS OF THE TERMITE GENUS ZOOTERMOPSIS

BY

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# RATE AND EXTENT OF DEVELOPMENT OF NEOTENIC REPRODUCTIVES IN GROUPS OF NYMPHS OF THE TERMITE GENUS ZOOTERMOPSIS

BY

S. F. LIGHT AND PAUL L. ILLG

## INTRODUCTION

A REMARKABLE characteristic of the termite colony is its ability to regulate the sexual function. In the normal wild colony still headed by the founding pair, this male and female are the only reproductives in the colony, but if one or both of them are killed or removed, or if groups of nymphs are isolated from them, some of the nymphs become precociously reproductive while still nymphal in many of their characteristics. These neotenic individuals are termed supplementary reproductives. Castle (1934) found that all nymphs of the damp-wood termites (*Zootermopsis* Emerson = *Termopsis* Hagen) above the third instar, except perhaps the late seventh-instar wing-padded nymphs, are capable of undergoing this precocious sexual development. The work of Light (1942–1943, 1944) has amply confirmed these findings.

The fact remains, however, that not all nymphs in any isolated group become reproductives; that is, the inherent tendency or capacity of nymphs to become neotenic reproductives is in some way controlled or regulated within the group (Light, 1943, p. 52). The relative number of nymphs in isolated groups of nymphs which do become supplementaries and the rate at which they appear within the groups differ widely, and the differences in rate and extent are, in part at least, correlated with differences in the origin of the nymphs, in the composition of the groups, and in the treatment which the groups receive.

A recently published review (Light, 1942–1943), summarizes briefly some of the differences in the rate and extent of reproductivity (the development of supplementaries and the resulting reproduction) found to occur between series of groups of isolated nymphs of the damp-wood termites. In this paper the experiments and results are presented in greater detail.

By "series" is meant a number of groups, usually 20, of the same origin and composition, set up at the same time and treated in the same way. The number of individuals in the group has most commonly been 20 but has varied from 10 to 50 with the experiment. Such series are arbitrarily designated from the beginning of the experiment, each group being given a group number. Thus, in experiment 3DS (p. 32) there were six identical series, 3DS-C1 to 3DS-C6, each composed of 20 groups. The groups of series of 3DS-C1 were designated 3DS-C1, 1 to 3DS-C1, 20 (table 17), and those of the other five series were similarly numbered.

## MATERIALS AND METHODS

The smaller damp-wood termite, *Zootermopsis nevadensis* (Hagen), has been used exclusively in the experiments here reported. The work of Heath (1927) and of Castle (1934), and all of our experience indicates, however, that the two closely related species of the genus, *Z. nevadensis* and *Z. angusticollis*, although they exhibit definite ecological and psychological differences and differences in developmental rates, are so similar in basic features of life cycle and development of supplementaries, and also in castes and constitution of colony in general, as to make it

safe to assume that findings obtained in studying one species will hold true for the other in these respects.

By far the greater part of the termites used, have been obtained from Monterey pine logs found in holding of the Del Monte Properties Company in Monterey County, California, and made available through the kindness of Mr. C. M. Olmsted, Division Manager. It may be remembered that the termites used by Heath (1907, 1927, 1928) in his studies on castes and colony development were obtained from this same region, as were some of those used by Castle (1934).

In collecting termites for experimental use, logs are partially opened in the field, and those which seem to contain thriving colonies are brought into the laboratory, where they are kept moist until they are needed. Invasions and mixing of colonies are found to occur if care is not taken to keep the logs separated.

The colony is not extracted until preparations for setting up the experiment are complete. When everything is ready, the log is sawed into short lengths and the termites shaken or jarred out on a paper and transferred at once to a collecting jar. Next the lengths of log are carefully split and the remaining termites removed to the collecting jar. Later the termites are separated from the debris and kept in culture dishes which contain moist paper and are kept closed as much as possible to prevent desiccation. Every effort is made to avoid rough handling and exposure to the drying effects of the atmosphere. Damaged individuals are discarded at once, since the dead become centers of development of fungus and bacteria which may then extend their attacks to the normal members of the group.

In the process of sorting the termites of large groups or colonies on the basis of types or instars, the group is usually placed in a clean glass dish. When this is tilted, the larger animals gradually move to the edge, where the experienced operator can make the selection and gently brush the individuals into separate dishes by means of a No. 6 camel's hair brush. All the dishes (usually half petri dishes) are constantly replenished with damp paper, which is cut as a rule in the form of a disc fitted to the bottom of the dish and, if need be, saturated with water to prevent the animals from crawling beneath the paper. Dishes not subject to immediate observation or manipulation are kept covered to retain moisture.

Individual termites are best handled by means of a small wooden spatula. Termites will usually leave a glass surface for the wood of the spatula. Forceps or similar instruments invariably bruise them. An individual termite can be removed from wood, paper, or glass by gently and carefully prying up with the tapered tip of the spatula until the animal loses its grip on the original surface, whereupon it will cling to the spatula, or by using the brush to push it against the spatula. Removal from the spatula is best accomplished by gentle brushing with the soft brush. In transferring termites in rapid, large-scale sorting or manipulations the habit is almost inescapably acquired of tapping the spatula sharply with the forefinger, to dislodge the termite. This is effective, but may seriously bruise the termite as it is flung into the dish unless the operator is sufficiently experienced to be able to judge closely the degree of force which may be safely exerted.

When the observations were to be made, the termites of a group were removed from the jar on the food material. From it they were gently brushed with a camel's hair brush either back into the housing jar or into a petri dish. When paper is the only food used, it is simple to brush all the termites into the jar, where the necessary observations can be made. If wood in fairly large pieces has been supplied, it is usually necessary to remove it from the jar, and often it must be split with a knife to remove all the animals from their galleries.

Castle (1934) used groups of 50 nymphs but only a few groups for each experiment. If the experiments gave clear-cut results, a few groups would suffice, but so far results have not been conclusive and the use of series consisting of many groups is indicated. Since it is imperative, because of the potential differences in reproductivity between colonies (brought out later in this paper), that all animals of a given experiment, controls as well as experimentals, be from a single colony, reduction of the group to the minimum number capable of functioning normally becomes especially important. Curiously, past evidence as to the smallest group able to accomplish integration and normal functioning has been conflicting. In some series, groups of 10 have prospered, but in general the impression holds that groups of 20 to 30 are more commonly successful. More evidence is called for here, since the differences may have been due more to moisture conditions than size of groups.

Each group was housed in a small ointment jar with a screw-top lid. Jars of one-, two-, or three-ounce capacity were used. Earlier experiments indicated that when large numbers are used in small containers, crowding offsets the advantage (if there is any) inherent in the larger group. Results of one such experiment set up in April, 1938, are given in table 1. The mortality after 20 weeks for groups of 30, 50, and 100 individuals, all in two-ounce jars, was, respectively 46 per cent, 50 percent, and 73 per cent, whereas that for groups of 5 and 10 in one-ounce jars was 26 per cent and 28 per cent, respectively, and that for groups of 20 in one-ounce jars was 50 per cent.

## MORTALITY IN EXPERIMENTAL GROUPS

All investigators who have attempted to use termites as experimental animals have encountered, and most have reported, the occurrence of relatively high mortality in experimental groups. This mortality seems to have various causes, some of them obvious as in the case of epidemics of disease, or where deaths are correlated with drying or with excessive moisture. The difficulties were well stated by Grassi and Sandias in speaking of *Kalotermes flavicollis,* "Some of these little nests can be kept alive for several months but many die after a few weeks. The tubes are partly filled with fragments of wood, which should be neither too dry nor too moist. In the former case, the insects gradually shrivel, contract, dry up and die; and in the latter case there is a deposit of water vapor on the inner walls, and they are evidently killed by over-dampness. Death ensues more or less rapidly according to the amount of water deposited, and is sometimes almost as sudden as if the insects were suffocated or chloroformed. Slow death due to over-dampness may be accompanied by distinct oedema or reddish discoloration of the body; the latter is accompanied by the presence of a bacterium which I have not investigated" (English translation, 1897).

When left to themselves in covered containers with plenty of food material (paper or wood), colonies, fractions of colonies, and even large mixed groups thrive, reproduce, and often increase in numbers. Under these circumstances the animals control their own moisture conditions and live essentially as they do in nature. For observational and experimental purposes, however, the use of smaller groups is necessary, and relatively frequent disturbance and handling are unavoidable. It was hoped that it would be possible to eliminate mortality during the course of experiments, or at least to reduce it to a negligible minimum. To this end the utmost care was exercised in handling the termites to avoid drying, squeezing, or otherwise injuring them. Various types of containers were tried, and various methods of supplying moisture. At times progress seemed to have been made, but it has not proved possible to eliminate sporadic deaths, as all tabulations of results will indicate. The nature and mode of occurence of these deaths are such as to leave their causes in doubt in

many instances. They continue to occur as long as the series is kept under observation, although certain groups in the series may show few if any deaths (thus no 20, table 17). Nearly always, also, some groups show very high mortality, rendering them useless for experimental purposes (thus no. 18, in table 17). Such groups behave in a fashion which suggests that the mortality is due to disease. In addition to the sporadic and incidental deaths throughout all the groups, and the epidemics localized within single groups, there are the epidemics of recognizable diseases which extend through many or all groups of a series or of all the series which come from a given colony. The most important of these diseases is the red disease mentioned by Grassi (1897, p. 251), and recently studied by DeBach and McOmie (1939). The *oedema* mentioned by Grassi seems to be a different disease, as is the second disease reported by DeBach and McOmie in which the head turns black. These, and at least one other characteristic disease, destroy large numbers of individuals, usually attack many groups in a series, and necessitate abandonment of the experiment. The diseases seem to be endemic and to become epidemic under conditions not yet understood by us, seemingly especially in experimental groups, probably because of the excessive handling to which they are unavoidably subjected.

In studies conducted since completion of the last of the experiments reported here we have developed methods of culture which greatly reduce the mortality in laboratory groups and which eliminate most of the abnormalities in laboratory conditions and treatment which led to the failure of reproductivity in many groups of the experiments. We hope to repeat enough of the experiments, using the new methods, to substantiate and amplify our conclusions and to clarify details. A repetition of all the experiments will be physically impossible.

## FOOD AND FEEDING

In some experiments the groups were fed moist filter paper; in others pieces of Monterey pine were used, covered with circles of filter paper or paper toweling. Coarse sawdust in 1.5- or 3-per cent agar is giving promising results under certain circumstances, as will be reported in later papers.

## RECORDS

At given intervals the group was removed from the jar, the population counted and recorded, and records made of the number of pigmented individuals (supplementaries) present, the presence of eggs, and, in some cases, the number of eggs, and the presence of young. Records were usually made for each group at weekly intervals beginning usually at the fourth or fifth week.

These data were compiled by a large staff closely supervised by trained scientists. The actual records, therefore, represent the routine observations of employees of different ability, training, and performance.

The items of raw data are of course subject to various inaccuracies such as mistakes in counting or recording, failure to recognize incipient supplementaries, and failure to record deaths of supplementaries. Supplementaries which were recorded as having died have been included in succeeding totals. Actually the dead individuals are usually eaten by the other members of the colony, or if the body is present it is usually so altered in appearance that its identification as a supplementary is difficult if not impossible. Where the record showed a decrease in the number of supplementaries in a group, though no specific record of death of supplementaries was made, it has been assumed that this decrease was due to the death of supplementaries if a corresponding decrease in recorded population made this a logical

assumption. Thus, if the number of supplementaries recorded for a given group at the tenth week was 4, and the surviving population 18, then a record of 3 supplementaries for that group for the eleventh week was taken to mean that one supplementary had died if the population was recorded as 17 or less, and the total supplementaries for the group was still considered as 4. On the original records this remains as 3, however. Then, if later the recorded number of supplementaries is increased to 4 again, it is assumed that a new supplementary has been produced. In some instances this may possibly be the recognition again of a previously recorded supplementary overlooked in a succeeding record.

In those instances in which, although a decreased number of supplementaries was recorded, there was no recorded loss in population of the group there was evidently an error of observation or of record. In such instances earlier and later records were scrutinized in order to determine whether the data indicated (1) that the preceding recorded number of supplementaries was in error, or (2) that the reduction in number of supplementaries was due to failure of the recorder to recognize one supplementary, or (3) that the recorded population was in error and a supplementary had actually died.

Unless otherwise stated, corrections have been made for all experiments reported on the basis outlined above. We believe that in general the effect of these corrections is to give a more accurate expression of reproductivity than that furnished by the uncorrected records. They have almost certainly resulted in a slight general exaggeration of the numbers of supplementaries produced. There is no reason to believe, however, that this trend is significant in degree or differentially concentrated by series.

Various procedures have been followed concerning groups which died out during the experiment. In future experiments sufficiently large series should be used to make it possible to completely disregard groups which die out during the experiment, or in which high mortality occurs, without endangering the validity of the conclusions.

Except for the corrections mentioned in the preceding paragraphs we present the findings of these experiments as they stand, in the belief that they indicate correctly major features and trends. They represent so great an investment of time that opportunity for similarly large-scale treatment of these problems will presumably not be available again. Determination of the details of the physiological and sociological events in groups of nymphs separated from reproductives calls for very time-consuming and detailed investigation. Such studies are necessary, however, to clarify the details of the situations here presented in rough outline.

## RECOGNITION OF SUPPLEMENTARIES

It is not always possible to recognize neotenic reproductives (supplementaries) by pigmentation, since this is at times delayed until after egg laying commences. In females, if the head is not sufficiently pigmented to allow for certain identification of the animal as a supplementary, the presence of a distinctly pigmented seventh sternite will confirm the diagnosis. With males the problem is more difficult, pigmentation being less striking and more irregular in its appearance in this sex. Here, in many instances at least, the anterior border of the last (ninth) sternite will show pigmentation in the functional male.

Ultimately all supplementaries become distinctly pigmented and in actual practice records are based entirely on pigmentation as observed with the aid of a reading lens with occasional reference to the pigmentation of the genital sternite. The con-

sistently lower in C3 with groups of 20 than in C4 with groups of 10. Again it will be seen, however, that the mortality was consistently higher in C3 than in C4. To compensate for the error which might arise from differential mortality, the percentage of existing population which had become supplementaries is given. Here, also, it will be seen that the percentage is distinctly lower for C3 than for C4, particularly in the later period of the experiment. More individuals became supplementaries in the larger groups of C3 than in the smaller groups of C4, but by no means twice as many. In other words, the group in some way regulates or determines the development of supplementaries.

It should be kept in mind, also, that development of supplementaries in a given group does not continue indefinitely at the initial rate. The groups tend to become stabilized in this regard at a figure varying to some extent with the series. The differences between series tend to remain the same, therefore, although the number of new supplementaries produced declines and a plateau is reached after about 8 to 10 weeks. The features of rate and extent of reproductivity are combined in practice by comparing the extent at any given time or series of times after isolation. Since the relative differences tend to persist throughout an experiment, they would seem to be expressions in part at least of the particular differences in physiological condition within the particular nymphs of the type or types used in the particular colony at the particular time under the particular conditions of treatment. As will be seen in experiments D2B and D4A (pp. 24–27) the potential reproductivity of a colony seems capable of shifting strikingly within a few weeks or months. The cause or causes of such shifts are not clear as yet, but are possibly of the same nature as those which result in the differences between colonies with respect to reproductivity.

## GENERAL RESULTS

By no means all of the different tests of the rate and extent of development of supplementary reproductives are reported here. Those are omitted in which there was an unusually high incidence of mortality, especially those in which great differences in mortality between controls and experimentals might have obscured differences in reproductivity. It may be said, however, that so far as the results of these unreported experiments are significant, they are in agreement with those presented. Further, the results of numerous experiments set up for other purposes are germane to this problem. These, again, are in agreement with those reported.

These experiments make it abundantly clear that differences in rate and extent of reproductivity are to be expected between series, however constituted. These differences and reproductivity as such are susceptible of quantitative formulation within the given experiment. Quantitative comparison of the findings of different experiments has not been possible. For one thing, the conditions of the several experiments (containers, food, handling) were significantly different. Dealing as we have been with a new laboratory animal and one presenting unusual difficulties because it is obligatorily social and closely adjusted to a biological regime not easily maintained in the laboratory, extensive experimentation with laboratory methods has had to go hand in hand with the more definitive experimental program. More important perhaps in preventing quantitative comparisons of results is the fact that groups of different composition were used in the experiments. This lack of uniformity was due partly to the fact that the ideal composition for such groups was itself a subject of experimentation, but more importantly to the very great differences in the composition of the colonies used as sources of material.

In some instances all of the animals used in an experiment were of the same instar.

This gives an apparent uniformity, but is far from the natural condition in the colony, where nymphs of various types and instars are associated. And the mere fact that nymphs are of the same instar does not mean that their potentialities for transforming into supplementaries are the same. Otherwise all of them would so transform, and at the same time, which is by no means the case.

In other experiments the nymphs were chosen at random. Even here, of course, there was really a certain amount of selection, since very small nymphs were seldom included, nor those late in the alate line (wing-padded nymphs with swollen wing pads). In still other experiments, after taking a census of the colony a formula was devised which would give each group a constitution similar to that of the colony as regards older nymphs at least. This system is illustrated in experiment 3DS reported on pages 32-36, below. The use of various nymphal types in making up the groups has become standard procedure since it makes it possible to derive a large number of groups from the same colony. It has not seemed important, however, to attempt to make the groups conform to the composition of the natural colony except as the preponderance of certain types of nymphs would naturally be reflected in the composition of the groups.

Even though groups identical in size and composition were used, and containers and treatment were identical, extensive quantitative comparisons would be difficult since each colony seems to possess a different inherent potential of reproductivity (p. 18). Perhaps if it seemed worth while the reproductivity of the control series of each experiment could be compared to a hypothetical normal and some coefficient of divergence be used which would allow for at least a rough over-all quantitative comparison. So far no attempt has been made in this direction.

The experiments here presented are given in what seems a logical order rather than in the order of their performance. In most instances only the summarized results by series are given, or significant examples, since the detailed records are voluminous. The picture of variation between groups of a series such as is brought out in tables 16 and 17 is largely lacking therefore, and it should be kept in mind that it was encountered in all experiments. Complete records are on file in the Department of Zoölogy of the University of California, Berkeley.

## DIFFERENCES IN REPRODUCTIVITY OF SERIES COMPOSED OF DIFFERENT NYMPHAL TYPES AND INSTARS FROM THE SAME COLONY

Pickens (1931) recognized that certain types of nymphs of the subterranean termites of the genus *Reticulitermes* develop more readily into supplementary reproductives than do others. Thus, he found that in *R. hesperus* supplementaries did not develop at all, or only after several months, in groups composed entirely of older apterous individuals, supposedly workers, whereas in mixed groups they usually appeared in from six to eight weeks. In agreement with these finding is the observation that by far the greater number of supplementary reproductives of *Reticulitermes* found in nature bear wing pads of varying lengths (Grassi, 1893; Snyder, 1918).

Castle (1934, p. 293) found, as did Heath (1927), that although both apterous and wing-padded (brachypterous) nymphs of *Zootermopsis angusticollis* do develop into supplementaries, the apterous types do so more readily, and that when both are present in a group the supplementaries produced are preponderantly apterous. According to Castle, the late seventh-instar brachypterous nymphs never develop into supplementaries. In *Zootermopsis*, in contrast to the conditions in

*Reticulitermes,* therefore, wing-padded supplementaries are rare (Heath, 1927; Castle, 1934; Light, 1942–1943). Our findings indicate that there are important differences between the instars and types of nymphs of *Zootermopsis* generally as to their reproductivity, and they allow for a tentative formulation of some of these differences.

These differences in potential reproductivity were so well known from laboratory and field experience that few definitive experiments were made to demonstrate and measure them. In fact, there are only three such experiments (ISC, 2DS and 6DS discussed below), and no one of these furnishes completely satisfactory information, for reasons which will become clear as the experiments are discussed.

TABLE 3

SIGNIFICANT RESULTS BY SERIES OF EXPERIMENT IS CARRIED OUT TO TEST DIFFERENCES IN REPRODUCTIVITY BETWEEN SERIES COMPOSED OF NYMPHS OF DIFFERENT INSTARS

| Series | Number of groups | Average number of supplementaries per group by weeks | | | | Average population per group by weeks | | | | Average, to nearest week, of weeks to first egg for groups for which eggs were recorded |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 4th | 8th | 12th | 16th | 4th | 8th | 12th | 16th | |
| Fourth instars | 4 | 1 | 2 3 | 3 0 | 3 5 | 20 5 | 17 8 | 11 3 | 9 3 | 10 |
| Fifth instars | 3 | 1 3 | 3 7 | 3 7 | 4 3 | 19 0 | 15 0 | 13 7 | 13 0 | 13 |
| Sixth instar apterous | 9 | 2 6 | 4 0 | 4 8 | 5 4 | 18 6 | 16 4 | 14 8 | 13 1 | 9 |
| Sixth instar brachypterous | 4 | 2 8 | 5 8 | 6 0 | 7 3 | 21 5 | 17 6 | 15 3 | 9 0 | 5 |
| Seventh instar apterous | 3 | 2 7 | 5 0 | 5 0 | 6 0 | 20 3 | 17 3 | 16 3 | 14 0 | 7 |
| Seventh instar brachypterous | 5 | 3 2 | 4 4 | 5 0 | 6 0 | 20 6 | 18 4 | 16 0 | 7 8 | 7 |

EXPERIMENT IS

The first such experiment, IS, was begun early in 1939, in the early period of the investigations, therefore, before supplementaries were distinguished in all cases with certainty and before certain refinements of observation and method were developed, and before the extent of the occurrence of differences due to chance was realized. The errors of record probably largely cancel one another but there remains the possibility that the number of groups in the series is so small (table 3) as to make the results of doubtful significance unless confirmed by more extensive series. Mortality (average population in table 3) was not strikingly different in the different series, certainly not enough so as to indicate any significant correlation between low reproductivity and high mortality, except perhaps for the series of fourth instars late in the experiment. The low population recorded for the two series made up of groups of brachypterous nymphs resulted in considerable part at least from the metamorphosis of alates in these groups, not from deaths. Alates are removed since they die under conditions of experimentation and if allowed to remain become centers of infection for the other members of the group. The colony used for the experiment was composed of 2,160 recorded individuals. It was headed by a number of supplementaries and included 92 soldiers.

One series (CS) consisting of 10 groups was set up on a formula corresponding to the general constitution of the colony. Two groups of this series died out before

the end of the experiment. Three others showed symptoms of the destructive red disease (see p. 3). Just why this series and not others should have been attacked is not clear. The results in this series are not presented because of the unfavorable conditions in the groups.

That portion of the colony remaining after series CS was set up was segregated by instars and set up in series of groups of 25 nymphs each (IS, table 3). Of third-instar nymphs there was only one full group, and a partial one of 12 nymphs. These are not included in the table since both had died out by the fifth week without producing recognizable supplementaries or laying eggs, presumably because younger nymphs require the care of older ones. Fourth instars made up 4 groups; fifth

TABLE 4

FREQUENCY DISTRIBUTION OF GROUPS IN SERIES OF EXPERIMENT IS

(At the twelfth week by per cent of number of groups of each series recorded as having a given number of supplementaries.)

| Series | Number of groups | Per cent of groups having various numbers of supplementaries | | | | | | | | Average number of supplementaries per group |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of supplementaries | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
| Fourth instars | 1 | | 25 | 50 | 25 | | | | | 3 0 |
| Fifth instars | 3 | | | 67 | | 33 | | | | 3 7 |
| Sixth instar apterous | 9 | | | 11 | 11 | 67 | 11 | | . | 4 8 |
| Sixth instar brachypterous | 1 | | | | | | 100 | | | 6 0 |
| Seventh instar apterous | 3 | | . | 33 | | 33 | | 33 | | 5 0 |
| Seventh instar brachypterous | 5 | | 20 | | 20 | 20 | 20 | | 20 | 5 0 |

instars, 3 groups; sixth-instar apterous, 9; sixth-instar brachypterous (wing-padded), 4; and seventh-instar brachypterous, 5. Records were begun at the fourth week, by which time supplementaries had appeared in all groups except the two composed of third-instar nymphs and in one group of fourth instars.

The seemingly significant feature of these results is an increase of reproductivity with the instar (tables 3 and 4), which is in line with our laboratory experience. The only series not in agreement with this generalization is that of the brachypterous sixth instars, which stands out as having distinctly the highest reproductivity however measured (tables 3 and 4), this in spite of the fact that alates metamorphosed in each of the groups of the series and that one group was recorded as diseased.

Another surprising feature of the results was the relatively high reproductivity of the series made up of groups of wing-padded (brachypterous) seventh-instar nymphs which is not at all in line with general laboratory experience. It seems very probable that careful distinctions were not made between brachypterous seventh instars (in the alate line and about to become alates) and either brachypterous sixth instars or brachypterous broad-heads, or both. The broad-headed nymphs, as is shown by the results of experiment 6DS, reported below, have a high potential of reproductivity, and the brachypterous sixth instars had, here at least, as already noted, a very high reproductivity.

EXPERIMENT 2DS

The significance of the nymphal composition of the group in determining its reproductivity was further illustrated by part of experiment 2DS, an experiment designed primarily to test colonies with respect to their relative potential reproductivity. Of interest in the present connection are three series from a colony of 2,400 nymphs,

TABLE 5

COMPOSITION OF GROUPS IN SERIES C5, C6, AND C7 OF EXPERIMENT 2DS, SERIES FROM THE SAME COLONY

| Series | Numbers of nymphs of different types in each group | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Broad-headed | Wing-padded | Seventh apterous | Sixth apterous | Fifth | Fourth | Soldiers | Total nymphs |
| 2DS-C5 . | 3 | 8 | 2 | 3 | 1 | 3 | 1 | 20 |
| 2DS-C6 | 7 | 0 | 3 | 4 | 2 | 4 | 0 | 20 |
| 2DS-C7 . | 0 | 7 | 3 | 4 | 2 | 4 | 0 | 20 |

TABLE 6

ESSENTIAL RESULTS BY SERIES, OF SERIES C5, C6, AND C7 OF EXPERIMENT 2DS

(Series of 20 groups per series, all from the same colony but of different nymphal composition; see table 5.)

| Data | Series | Weeks after setting up experiment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 4th | 5th | 6th | 7th | 8th | 9th | 10th | 11th |
| Number of groups in which supplementaries were recorded | 2DS-C5 | 7 | 8 | 10 | 11 | 11 | 12 | 12 | 12 |
| | 2DS-C6 | 14 | 17 | 19 | 19 | 20 | 20 | 20 | 20 |
| | 2DS-C7 | 9 | 10 | 10 | 10 | 11 | 11 | 12 | 12 |
| Total of supplementaries produced | 2DS-C5 | 10 | 15 | 19 | 20 | 20 | 21 | 21 | 21 |
| | 2DS-C6 | 25 | 39 | 45 | 47 | 49 | 49 | 51 | 52 |
| | 2DS-C7 | 11 | 12 | 11 | 14 | 15 | 15 | 16 | 16 |
| Number of groups in which eggs were recorded | 2DS-C5 | 1 | 1 | 2 | 2 | 3 | 6 | 7 | 8 |
| | 2DS-C6 | 1 | 2 | 7 | 11 | 11 | 17 | 17 | 18 |
| | 2DS-C7 | 3 | 3 | 5 | 6 | 6 | 7 | 7 | 9 |
| Number surviving in each series from the original 400 | 2DS-C5 | 380 | 375 | 371 | 368 | 364 | 363 | 358 | 352 |
| | 2DS-C6 | 377 | 369 | 352 | 342 | 338 | 337 | 333 | 330 |
| | 2DS-C7 | 385 | 383 | 378 | 367 | 363 | 360 | 358 | 356 |

200 alates, and 28 soldiers, and headed by a primary pair. These three series, each of 20 groups of 20 nymphs, were set up late in November, 1940. Table 5 gives the composition of these groups in terms of types of nymphs as recognized at that time. The broad-headed nymphs were apterous nymphs of the eighth or later instars. The wing-padded nymphs probably included brachypterous sixth- and seventh-instar individuals and even some of the later instar (the "wing-padded broad-heads" of 6DS); the records are not precise on this point.

Soldiers were present only in series 2DS-C5, one in each group. They do not enter into the records given in table 6 and there is no reason to believe that they affected

the reproductivity of their groups. It is possible, however, that they did exert an inhibitory influence on soldier development, since, whereas only one soldier developed in 2DS-C5, three developed in 2DS-C6 and four in 2DS-C7, in three different groups of each series. Although the records are not explicit, it seems probable that these soldierlike individuals were supplementaries, neotenic-soldier intergrades, the reproductive soldiers of Heath (Light 1942–1943).

It may be noted in passing that the viability was high for the soldiers in the groups of series 2DS-C5. Only two, or 10 per cent, of them had died by the end of

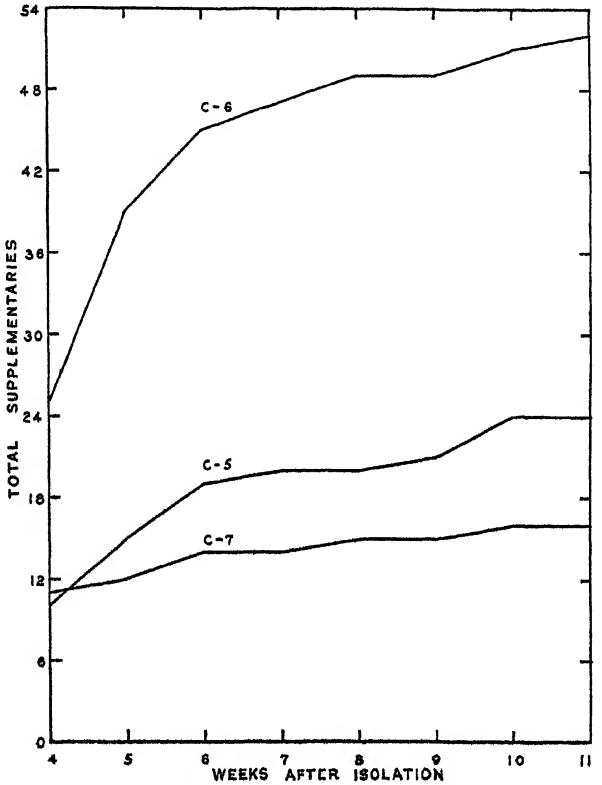


Fig. 1. A graphic expression of rate and extent of reproductivity (total supplementaries by weeks) for series C 5, C 6, and C 7 of experiment 2DS. The nymphs used in the three series were from the same colony but the composition of the groups differed in each series (see table 5).

The groups of series C 6, with the highest reproductivity, were without wing-padded nymphs and included eight broad headed nymphs, whereas the groups of C-5 and C-6 contained respectively eight and seven wing padded nymphs and C-5 contained only three broad-headed nymphs per group and C 6 none.

the eleventh week. Nor was there any indication that the presence of the soldiers was unfavorable to these groups. The mortality of nymphs in these groups was only 12 per cent. It was slightly less, 11 per cent, in 2DS-C7, but considerably more, 17.5 per cent, in 2DS-C6.

Table 6 and figure 1 give the essential results with respect to reproductivity. 2DS-C6 stands out as having much the highest reproductivity of the three series whether measured in terms of groups with supplementaries, total supplementaries produced, or groups with eggs. Figure 1 brings out the striking difference in total supplementaries produced. Series 2DS-C6 differed significantly in its constitution

from both 2DS-C5 and 2DS-C7 in that it lacked wing-padded nymphs, of which the groups of the other two series contained a very considerable number, 8 and 7, respectively, and in that its groups included 7 broad-headed nymphs as against 3 in 2DS-C5 and none in 2DS-C7.

With respect to mortality, or viability, conditions were unusually good in these series (see table 6). Of the 60 groups only one suffered a loss by death of as much as 35 per cent, and the average mortality at the eleventh week in the series which had the highest mortality (2DS-C6) was only 17.5 per cent. As for reproductivity, however, it will be seen that, whereas all groups of 2DS-C6 had supplementaries by the eighth week and all but two had eggs by the eleventh week, 8 groups in 2DS-C5 and 8 in 2DS-C7 had produced no supplementaries by the eleventh week, and for 12 groups in C5 and 11 in C7 no eggs had been recorded.

Viability was good in all these groups, better indeed in the two with low reproductivity than in 2DS-C6. One obvious difference as already mentioned, between the groups of the series with unusually low reproductivity and those of 2DS-C6 was the presence in the former of numerous wing-padded nymphs and their absence in 2DS-C6. It might be postulated that the wing-padded nymphs in some way inhibited reproductivity in the groups of the two series containing them. It has long been common laboratory knowledge that wing-padded nymphs of the seventh instar are slow to become supplementaries (although this was not true in experiment IS above). Some of the relative difference in reproductivity between series in 2DS is probably to be attributed to the presence in the groups of 2DS-C6 of numerous broad-headed nymphs and their absence in 2DS-C7 and their scarcity in 2DS-C5. The supplementaries most commonly encountered in nature are broad-headed and laboratory experience indicates that broad-headed nymphs are quick to become supplementaries (see also 6DS below). Unfortunately no record was kept of the types of nymphs which became supplementaries in these series. A record was made at the twelfth week of the number of broad-headed nymphs surviving as such in the groups of 2DS-C5, each of which originally contained 3 broad-headed nymphs. In 3 groups all 3 broad-headed nymphs were recorded absent, in 9 groups only 1 was recorded present at the twelfth week, in 8 groups 2 were recorded as such, and in 1 group all 3 were recorded as present. If we consider the unrecorded broad-heads as having died, the average recorded mortality of broad-heads would be 53 per cent as against a general mortality of only 12 per cent for the series. It is probable, therefore, that many of these broad-heads had become supplementaries. If this assumption is correct, it would seem probable that the differences in reproductivity between these series are to be explained, in part at least, on the basis of the presence or absence of broad-headed nymphs or the relative numbers of such nymphs present.

### EXPERIMENT 6DS

The last experiment relating to differences in reproductive potential of the different types of nymphs in a colony, experiment 6DS, was performed early in 1942, with nymphs from the colony a part of which was used in experiments EX, SE, SX, and EX on extract inhibition of reproductivity (Light, 1944). This colony consisted of more than 12,000 nymphs and was headed by a large number of supplementary reproductives, including many neotenic-soldier intergrades. It was peculiar also in that it included a large number of wing-padded nymphs of late instar, chiefly wing-padded (brachypterous) broad-heads. For experiment 6DS 2,400 nymphs were used in six series of 40 groups of 10 nymphs each. The constitution of the groups of the different series is given in table 7.

TABLE 7

COMPOSITION OF GROUPS OF THE SERIES OF EXPERIMENT 6DS

| Series | Wing-padded (brachypterous) broad-heads | Eighth-instar apterous broad-heads | Seventh-instar apterous | Wing-padded (brachypterous) sixth and seventh | Sixth-instar apterous | Fifth instar |
|---|---|---|---|---|---|---|
| 6DS-6 | | | | | 10 | |
| 6DS-7 | | | 10 | | | |
| 6DS-BH | | 10 | | | | |
| 6DS-BHWP | 10 | | | | | |
| 6DS-A | | 2 | | 4 | 2 | 2 |
| 6DS-B | | 2 | 4 | | 2 | 2 |

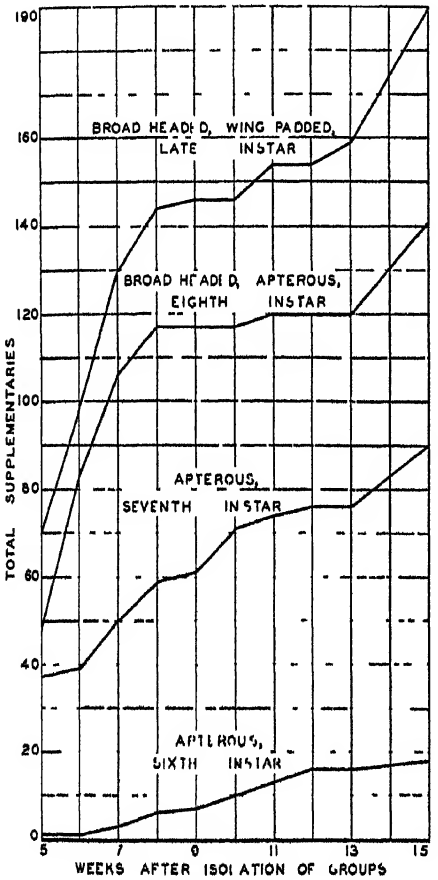


Fig. 2

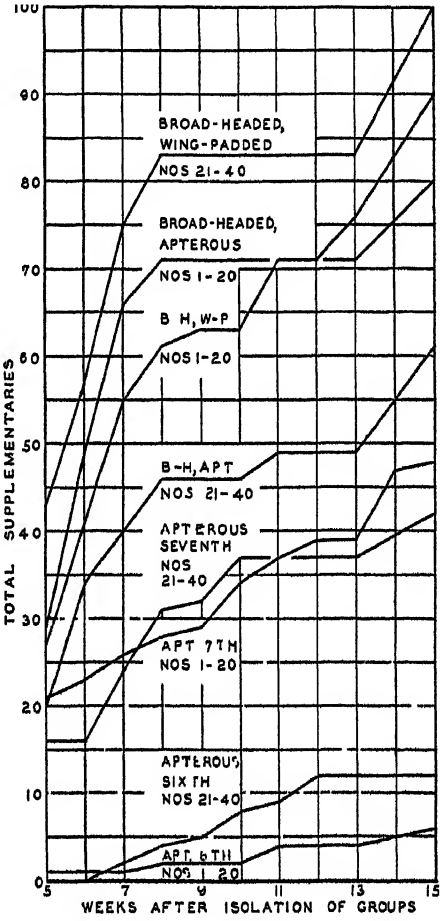


Fig. 3

Fig. 2. Supplementaries by weeks for the four homogeneous series of 6DS showing increase in reproductivity with age of instar.

Fig. 3. Supplementaries by weeks for the four homogeneous series of 6DS (see fig. 2) but with the first 20 and last 20 groups of each series presented as separate series. (See the discussion, pp. 16 and 32.)

Figure 2 gives the results in terms of total supplementaries produced by the four homogeneous series from the fourth through the fifteenth weeks. The same results are presented in figure 3 but each series is divided into two subseries, one consisting of groups 1 to 20, the other of groups 21 to 40. See page 32 for a discussion of the situation thus recorded. Table 8 gives the frequency distribution of groups in each of the series by numbers of supplementaries recorded by the fifteenth week. Table 9

TABLE 8

FREQUENCY DISTRIBUTION OF GROUPS OF SERIES OF EXPERIMENT 6DS

(By numbers of supplementaries produced by the fifteenth week.)

| Series | Number of supplementaries | | | | | | | | Number of groups | Average number of supplementaries per group |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| Wing-padded broad-heads | | | 2 | 4 | 10 | 14 | 7 | 3 | 40 | 4 70 |
| Apterous broad-heads | | 2 | 10 | 9 | 9 | 6 | 3 | 1 | 40 | 3 50 |
| Combination A | 1 | 2 | 6 | 17 | 9 | 5 | | | 40 | 3 20 |
| Combination B | 2 | 8 | 17 | 10 | 3 | | | | 40 | 2 10 |
| Seventh-instar apterous | 2 | 4 | 21 | 6 | 4 | 1 | 1 | | 39 | 2 33 |
| Sixth-instar apterous | 27 | 8 | 5 | | | | | | 40 | 0 15 |

TABLE 9

EXPERIMENT 6DS

| Series | Per cent of groups with supplementaries by weeks | | | | | Per cent of groups with eggs by weeks | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 4th | 5th | 6th | 8th | 10th | 4th | 6th | 10th | 15th |
| BH-WP . . | 62 5 | 82 4 | 87 1 | 100 0 | 100 0 | 7 5 | 32 5 | 90 0 | 97 5 |
| BH-apterous . | 45 0 | 65 0 | 87 5 | 97 5 | 97 5 | 5 0 | 10 0 | 85 0 | 100 0 |
| Combination A | 62 5 | 80 0 | 85 0 | 95 0 | 97 5 | 0 | 7 5 | 75 0 | 90 0 |
| Combination B .. | 55 0 | 57 3 | 65 0 | 77 5 | 85 0 | 5 0 | 27 5 | 70 0 | 82 5 |
| Seventh-apterous | 40 0 | 45 0 | 52 0 | 70 0 | 87 5 | 0 | 5 0 | 70 0 | 85 0 |
| Sixth-apterous | 0 | 2 5 | 2 5 | 12 5 | 20 0 | 0 | 0 | 17 5 | 40 0 |

gives results in terms of per cent of groups in which supplementaries had developed and per cent of groups in which eggs had been observed by particular weeks. However formulated, the recorded reproductivity is greatest in the series consisting of wing-padded broad-heads and very much the lowest in that series made up of apterous sixth-instar nymphs. As is shown by table 10, the mortality was not high, nor did it vary sufficiently between series to explain the differences in reproductivity, unless perhaps in the series consisting of apterous seventh-instar nymphs, which actually suffered the highest mortality since one group died out entirely. The series of sixth-instar apterous nymphs which had the lowest reproductivity suffered nevertheless the least mortality.

It will be noted from table 7 that the only difference in constitution between series A and B, which were made up of composite groups, was that each group in A contained 4 wing-padded nymphs and no seventh-instar apterous nymphs, whereas

each group of B contained 4 apterous seventh-instar nymphs and no wing-padded ones. General laboratory experience, not quantitatively based as yet, leads us to expect that wing-padded nymphs would be slow to become supplementaries if, indeed, they do not actually inhibit or retard the development of other nymphs into supplementaries, as might possibly be the case in experiment 2DS reported above (p. 14). In 6DS, however, combination A which included wing-padded nymphs showed a consistently higher reproductivity than did combination B in which wing-padded nymphs were replaced by apterous seventh-instar nymphs. Further, the reproductivity of B is similar to that of the series made up entirely of seventh-instar apterous nymphs (tables 8 and 9). In a series of graphs in which total supplementaries is plotted against elapsed time, the graph for series A would fall close together throughout, with that for apterous broad-headed nymphs and that for series B close to that for the series of apterous sixth instars (see fig. 2). The

TABLE 10

MORTALITY IN THE SERIES OF EXPERIMENT 6DS AT THE FIFTEENTH WEEK

| Series | Per cent mortality in existing groups | Number of groups existing | Existing groups with at least 50 per cent mortality |
|---|---|---|---|
| Wing-padded broad-heads | 13 75 | 40 | 0 |
| Apterous broad-heads | 16 25 | 40 | 0 |
| Combination A | 12 75 | 40 | 1 |
| Combination B | 16 25 | 40 | 0 |
| Seventh apterous | 15 75 | 39 | 3 |
| Sixth apterous | 11 75 | 40 | 1 |

only explanation for this behavior suggested by the available data is the fact that the series of apterous seventh-instar nymphs had high mortality (see table 10), possibly because of disease acquired during the original segregation preliminary to setting up the experiment. One of its groups died out completely and three others had more than 50 per cent mortality. It seems possible that the groups of the composite series B were infected by the apterous seventh-instar nymphs and that the reproductivity of the two series whose groups contained seventh-instar nymphs was far below normal for this instar.

In general it will be observed that our belief in the existence of characteristic differences in the potential reproductivity of nymphs of the various types and instars is based on a small amount of evidence, some of which is somewhat conflicting, because of special experimental conditions. Despite this fact, however, the following tentative conclusions seem justified, subject to further experimental verification: (1) Different instars in a given colony have different potentialities as regards reproductivity. (2) Potential reproductivity increases in general with the progressive age of instars. (3) The broad-headed nymphs, which are of eighth or later instars, become supplementaries earliest and are the most vigorously reproductive. Two further conclusions seem tentatively justified although less well based than the preceding ones: (1) apterous nymphs in general show a greater tendency toward neoteny than do brachypterous nymphs of the same instar, (2) there is a possibility that the presence of a considerable number of seventh-instar wing-padded nymphs tends to reduce the rate and extent of development of associated nymphs into supplementaries.

## DIFFERENCES IN POTENTIAL REPRODUCTIVITY BETWEEN COLONIES

### EXPERIMENT D2

D2, the first experiment designed to determine whether there are differences in potential reproductivity between colonies was begun in December, 1937. Ten colonies of *Z. nevadensis* were used. A series of 20 groups of 10 nymphs of the same instar, supposedly the fifth, was set up from each colony. Groups which died out during the experiment were not considered in compiling the data. Methods of handling were not well developed at this time, mortality was high and irregular and hence the results obtained should be considered as only tentatively significant. They are in line with other results, however, and, as will be pointed out more in detail later, there seems no significant correlation between high mortality and low reproductivity where series are concerned.

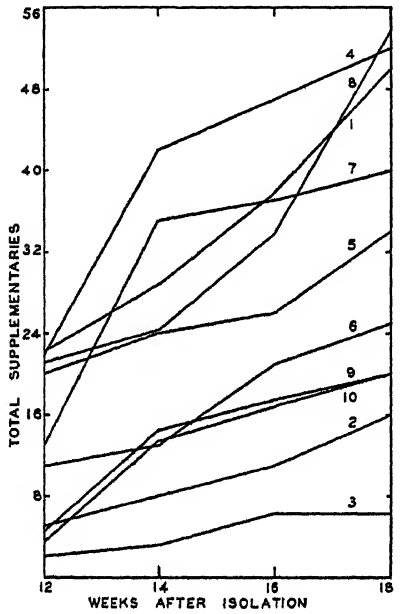


Fig. 4. Experiment D2. Reproductivity in ten series, each from a different colony. Reproductivity is presented as total supplementaries produced by the surviving groups of each series at the twelfth, fourteenth, sixteenth, and eighteenth weeks and expressed in terms of 20 groups per series.

Significant samples of the results are presented in table 11 and in figures 4 and 5. The differences in reproductivity between colonies are seen to be very considerable, greater presumably than can be expected to result from random differences in composition and treatment. For series D2-3, for example, with the lowest reproductivity, supplementaries were recorded in only 5 groups, a total of 5 supplementaries at the eighteenth week, whereas in D2-8 supplementaries were produced in all 20 groups, and the total was 51. For D2-3, further, eggs had been recorded in but three groups by the eighteenth week, and a total of only 14 eggs, whereas in D2-5, for example, a total of 151 eggs was produced by 16 groups; and in D2-8, which seems to have shown the highest reproductivity, in general, eggs were recorded in 16 groups, the total recorded being 121.

When an attempt is made to rank these 10 series according to reproductivity (table 11) D2-3 clearly ranks lowest and D2-8 highest. Series 10, 9, and 2 rank low, but their relative rank is not clear. Series 4 and 1 are high, and series 7, 5, and 6 are intermediate.

There are considerable differences between the series of D2 with respect to recorded mortality (table 11), and these differences might be thought to be causally related to the differences in reproductivity or at least indirectly correlated with them. There can be little doubt that there is a negative correlation between reproductivity and mortality in certain groups of many experiments. Perusal of table 11 will make apparent, however, what seems to hold in all such experiments, that is, a lack of correlation between high mortality and low reproductivity when series

TABLE 11

EXPERIMENT D2, CONSISTING OF TEN SERIES RUN CONCURRENTLY, EACH FROM A DIFFERENT COLONY

(Data are given only for groups surviving the experiment and in terms of surviving groups. Figures are for the eighteenth week. The series are arranged approximately in order of decreasing reproductivity.)

| Series | Per cent of groups with eggs | Per cent of groups with supplementaries | Average number of supplementaries per group | Groups dying out | Per cent of mortality for surviving groups | Rank on basis of mortality |
|---|---|---|---|---|---|---|
| 8 | 75 | 100 | 2 5 | 0 | 35 5 | 4 |
| 4 | 74 | 95 | 2 7 | 1 | 26 9 | 9 |
| 1 | 67 | 94 | 2 3 | 2 | 46 1 | 1 |
| 7 | 68 | 100 | 2 0 | 1 | 24 2 | 10 |
| 5 | 80 | 75 | 1 6 | 0 | 30 5 | 7 |
| 6 | 25 | 85 | 1 2 | 0 | 33 5 | 5 |
| 10 | 32 | 58 | 0 9 | 1 | 29 0 | 8 |
| 9 | 17 | 56 | 0 9 | 2 | 40 6 | 2 |
| 2 | 25 | 60 | 0 8 | 0 | 36 0 | 3 |
| 3 | 16 | 26 | 0 3 | 1 | 32 6 | 6 |

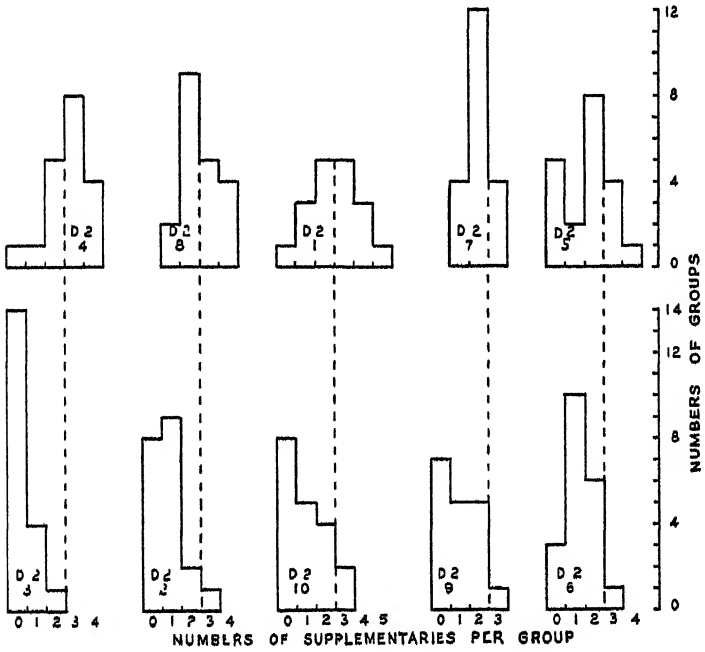


Fig. 5. Histograms showing frequency distribution of groups of the ten series of experiment D2 (each series from a different colony) by numbers of supplementaries at the eighteenth week. The histogram of the series with the greatest number of groups having three or more supplementaries is placed at the left above that of the series with the lowest reproductivity thus measured, the second highest over the second lowest, and so on. The dotted vertical line marks the boundary between two and three supplementaries per group in each instance.

are considered. For example, the series with the highest mortality, D2-1, and that with the lowest mortality, D2-7, were approximately equal in reproductivity at the sixteenth week and not far apart at the eighteenth and the colony with the lowest reproductivity, D2-3, suffered only about average mortality, less indeed than D2-8, which ranked highest in reproductivity.

EXPERIMENT 1DS

The next experiment was designated 1DS. It was carried out in the spring of 1940, with six series from as many different colonies. Each series consisted of 20 groups, each composed of one soldier and 20 nymphs chosen at random. Weekly records

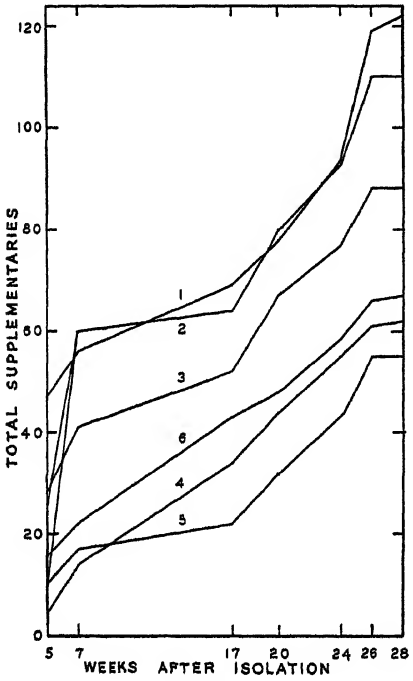


Fig. 6

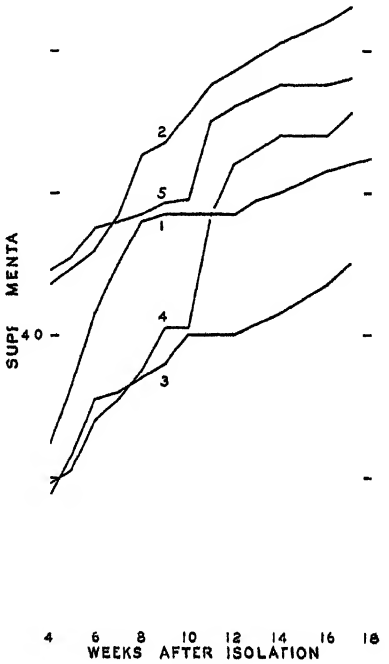


Fig. 7

Fig. 6. Experiment 1DS. Six series each from a different colony. Points represent numbers of supplementaries per series for the fifth, seventh, seventeenth, twentieth, twenty-fourth, twenty-sixth, and twenty-eighth weeks.

Fig. 7. Supplementaries weekly by series for experiment 4DS; five series of the same constitution each from a different colony. See also fig. 16, *a*.

were made as follows: for the fourth to seventh weeks, inclusive, for the seventeenth to twenty-second weeks, inclusive, the twenty-fourth to twenty-sixth weeks, inclusive, and, finally, for the twenty-eighth week. Figure 6 gives the results in terms of total supplementaries for selected weeks. The records show similar differences when reproductivity is measured in terms of groups with supplementaries, groups with eggs, or groups with young. Series 1 and 2 were consistently higher for all three criteria, series 5 and 4 consistently low, and 6 and 3 intermediate. Here, as in D2, high reproductivity showed no consistent correlation with low mortality or vice versa when series are compared. Series 5, which was consistently lowest in reproductivity from at least the seventeenth week, was consistently lowest, also,

in mortality (46.25 per cent), with all groups surviving at the twenty-eighth week. This is in contrast to a mortality of 52 to 68 per cent in the other series, whose reproductivity greatly exceeded that of series 5. It should be remembered that this experiment was continued for a much longer period than any other. At the seven-

TABLE 12

SUMMARY OF RESULTS OF EXPERIMENT 4DS

(Five series of groups of the same composition, each series from a different colony. Series are listed in the order of increasing reproductivity.)

| | Series number | | | | |
|---|---|---|---|---|---|
| | C3 | C4 | C1 | C5 | C2 |
| Fourth week | | | | | |
| Per cent of groups with supplementaries | 70 | 60 | 65 | 95 | 90 |
| Average supplementaries per group | 0 85 | 0 95 | 1 20 | 2.40 | 2 35 |
| Per cent of groups with eggs | 10 | 15 | 5 | 5 | 30 |
| Sixth week | | | | | |
| Per cent of groups with supplementaries | 85 | 70 | 85 | 95 | 100 |
| Average supplementaries per group | 1 55 | 1 40 | 2.15 | 2 75 | 2 6 |
| Per cent of groups with eggs | 35 | 65 | 65 | 65 | 75 |
| Eighth week | | | | | |
| Per cent of groups with supplementaries | 85 | 75 | 95 | 100 | 100 |
| Average supplementaries per group | 1 70 | 1 65 | 2.80 | 2 85 | 3 25 |
| Per cent of groups with eggs | 50 | 80 | 80 | 90 | 75 |
| Tenth week | | | | | |
| Per cent of groups with supplementaries | 90 | 80 | 95 | 100 | 100 |
| Average supplementaries per group | 2 00 | 2 05 | 2 05 | 2 95 | 3 50 |
| Per cent of groups with eggs | 75 | 90 | 95 | 95 | 100 |
| Twelfth week | | | | | |
| Per cent of groups with supplementaries | 90 | 100 | 95 | 100 | 100 |
| Average supplementaries per group | 2 00 | 3 20 | 2 85 | 3 60 | 3 85 |
| Per cent of groups with eggs | 80 | 95 | 95 | 100 | 100 |
| Eighteenth week | | | | | |
| Per cent of groups with young | 30 | 50 | 65 | 45 | 75 |
| Per cent of groups with supplementaries | 95 | 100 | 100 | 100 | 100 |
| Average supplementaries per group | 2 50 | 3 55 | 3 25 | 3 80 | 4.30 |
| Per cent of groups with eggs | 95 | 95 | 100 | 100 | 100 |
| Number of alates | 0 | 28 | 32 | 26 | 25 |
| Per cent of mortality | 32 5 | 19 25 | 11 | 23 | 31 |

teenth week mortality was much lower than the percentages noted above and was in approximate agreement with that in other experiments.

The fact that nymphs were chosen at random rather than being all of one type or according to a given formula adds an additional uncontrolled factor. However, since the differences in reproductivity between series each from a different colony are markedly greater than those between identical series from the same colony run

at the same time (see figs. 6 and 14) and since the existence of such difference is in agreement with the results of other experiments (D2 and 4DS) in which all groups were of the same composition, these results must be considered as adding to the evidence for the existence of differences between colonies with respect to potential reproductivity.

EXPERIMENT 4DS

A final experiment, 4DS, was begun in June, 1941, in an effort to obtain decisive evidence about the existence of differences in potential reproductivity between colonies. Each of five series was from a different colony, and consisted of 20 groups of 20 nymphs each. Each group had the same approximate formula as regards nymphal types, namely, 3 wing-padded nymphs, 4 apterous nymphs of the seventh instar, 4 apterous nymphs of the sixth instar, 4 of the fifth instar, 3 of the fourth instar, and 2 of the third instar. In one series, 4DS-C3, the wing-padded nymphs were all of the sixth instar with very small wing pads; in the other series they were brachypterous nymphs chosen at random, probably chiefly seventh instars, with possibly some broad-headed wing-padded nymphs. That they were chiefly seventh instars is shown by the fact that many alates appeared in all series except 4DS-C3 (table 12). Reduced reproductivity would have been expected in the groups producing alates. Contrary to expectations, however, 4DS-C3 had by far the lowest reproductivity (fig. 7 and table 12) and the highest mortality (table 12). The low reproductivity in this series may of course be directly or indirectly correlated with the high mortality. 4DS-C2, however, which was well ahead in reproductivity from the seventh week on, experienced the highest mortality if alate development is counted as death, and much higher than any series except C3 if alates are not counted as deaths.

NATURE OF FACTORS CAUSING DIFFERENCES IN REPRODUCTIVITY

In table 13 the series constituting the experiments D2, 2DS, 4DS, and 1DS are listed. In each experiment the series are listed in an order approximating increasing reproductivity, and in separate columns the constitutions of the colonies of origin are given, so far as known, in order to determine whether there is any obvious correlation between the degree of reproductivity and any known feature of constitution of the colony.

There seems no correlation between relative numbers of soldiers and reproductivity. For example colony D2-8 having the highest relative number of soldiers in D2, 11.7 per cent, had highest reproductivity; whereas D2-2 with next to the highest number of soldiers, 9.2 per cent, was next to the lowest in reproductivity. In 2DS the colony with the lowest proportion of soldiers was intermediate in reproductivity, the next was the lowest in reproductivity, and the one with the third lowest soldier ratio was the highest in reproductivity.

The two nymphal categories recorded, apterous and wing-padded, are so broad as to be of little significance. As previously shown, some of the broad-headed nymphs which show by far the greatest reproductivity (see p. 17) would fall into the wing-padded group and some into the apterous group.

The only feature which suggests itself as causally related to differences in reproductivity is the nature and number of the reproductives heading the colony. For example, it will be seen that the series in each of the four experiments which had the lowest reproductivity was from a colony headed by a primary queen, presumably by a primary pair, although the king was recovered in only one instance. On the other hand, the series in each of the four experiments which had the highest repro-

There is good reason from laboratory experience to believe that colonies headed by supplementaries are more erratic with regard to reproductivity than those headed by primaries, although quantitative proof is not available. The nymphs of

TABLE 13

RELATION BETWEEN REPRODUCTIVITY OF NYMPHS IN ISOLATED GROUPS AND THE COMPOSITION OF THE COLONY OR ORIGIN OF THE GROUPS

(Series in each experiment are listed approximately in order of increasing reproductivity.)

| Series | Reproductives | Soldiers | Wing-padded nymphs | Apterous nymphs |
|---|---|---|---|---|
| D2-3[b] | (King[a]) Queen | 232 | 2,000 | 3,000 |
| D2-2[b] | King (Queen) | 102 | 200 | 800 |
| D2-9[b] | King (Queen) | 45 | 300 | 1,500 |
| D2-10 | 35 supplementaries | 200 | 500 | 425 |
| D2-6[b] | King, Queen | 7 | 2,000 | 2,200 |
| D2-5 | 4 supplementaries | 42 | 0 | 500 |
| D2-7 | ? | ? | ? | ? |
| D2-1 | King (Queen) | 53 | 3 | 650 |
| D2-4 | King, Queen | 38 | 0 | 505 |
| D2-8 | 5 supplementaries | 40 | 0 | 300 |
| 2DS-C5, 2DS-C6, 2DS-C7 | (King), Queen | 28 | 1,008 | 1,392 |
| 2DS-C3 | 17 supplementaries | 81 | 912 | 1,368 |
| 2DS-C2 | 16 supplementaries | 60 | 248 | 462 |
| 2DS-C10 | 21 supplementaries | 27 | 527 | 2,247 |
| 2DS-C1 | 4 supplementaries | 106 | 686 | 1,020 |
| 2DS-C4 | (King), Queen | 82 | 288 | 912 |
| 2DS-C8 | 19 supplementaries | 21 | 222 | 545 |
| 4DS-C3 | (King), Queen | 117 | 660 | 2,424 |
| 4DS-C4 | 18 supplementaries | 160 | 492 | 3,705 |
| 4DS-C1 | (King), Queen | 128 | 1,436 | 3,080 |
| 4DS-C5 | 12 supplementaries | 160 | 931 | 1,518 |
| 4DS-C2 | 126 supplementaries | 132 | 3,767 | 2,562 |
| 1DS-C5 | King, Queen | 235 | 112 | 3,280 |
| 1DS-C4 | 9 supplementaries | 93 | 308 | 1,231 |
| 1DS-C6 | King, (Queen) | 22 | 601 | 594 |
| 1DS-C3 | (King), Queen | 85 | 359 | 1,537 |
| 1DS-C2 | 5 supplementaries | 20 | 1 | 527 |
| 1DS-C1 | 4 supplementaries | 87 | 161 | 779 |

[a] King or queen in parentheses indicates that the reproductive in question, although not actually found, was assumed to have been present.

[b] Numerous supplementaries were recorded for the colony of origin. Since these colonies were headed by primary reproductives, these reported supplementaries are assumed to have been broad-headed nymphs which were not distinguished from supplementaries at the time.

the colonies headed by primaries have been inhibited throughout their entire nymphal life, whereas in those colonies headed by supplementaries the nymphs which have persisted from the time of the death of the primaries must have been freed from inhibition for at least the period immediately succeeding the death of the primaries. Furthermore, there is some evidence (Light 1942–1943) that primaries are more effective in contact inhibition than are supplementaries. Supposedly, therefore, the nymphs of supplementary-headed colonies might be expected

to be less inhibited than those of primary colonies. In some instances the contrary seems to be true, however, and this could be readily explained, theoretically, in the case of most of all nymphs of colonies headed by many supplementaries if it be supposed that the death of the primaries and the resulting uninhibited period occurred at some relatively distant time and that the young nymphs have developed subsequently in the presence of many reproductives and are, therefore, strongly inhibited. This seems the only obvious explanation for the very low reproductivity of the series 6DS-6 (see page 16 and fig. 2 and tables 8 and 9).

## SERIES FROM THE SAME COLONY RUN AT DIFFERENT TIMES

Questions which present themselves here are whether the differences in potential reproductivity between colonies remain constant or whether they shift with the season, with the period in the cycle of the colony, or with the composition of the colony which in turn may be supposed to shift both with the season and with the cycle of the colony and perhaps because of chance incidence of factors as yet undetermined.

TABLE 14

EXPERIMENT D2-B

(Series arranged in order of decreasing reproductivity.)

| Series | 4th | 6th | 8th | 10th | 12th | 14th | 16th | 18th |
|---|---|---|---|---|---|---|---|---|
| | Groups containing supplementaries by weeks as per cent of surviving groups | | | | | | | |
| D2-3B | 100.0 | 100.0 | 100 0 | 100 0 | 100 0 | 100 0 | 100 0 | |
| D2-9B | 27 3 | 63 6 | 81 8 | 90 9 | 100 0 | 100 0 | 100 0 | |
| D2-8B | 10 0 | 55 0 | 68 4 | 83 3 | 88 9 | 94 4 | 100 0 | |
| D2-8+10B | 5 3 | 15.7 | 57 9 | 68 4 | 83 3 | 100 0 | 100 0 | |
| | Total supplementaries produced by weeks as per cent of original population of surviving groups | | | | | | | |
| D2-3B | 13 8 | 18 8 | 23 8 | 26 3 | 28 6 | 37 1 | 37 1 | 40 0 |
| D2-9B | 5 5 | 13 6 | 19 1 | 21 8 | 25 5 | 27 3 | 28 2 | 33 6 |
| D2-8B | 1 0 | 7 0 | 8 9 | 12 1 | 14 4 | 16 1 | 18 8 | 23 8 |
| D2-8+10B | 0 5 | 2 1 | 8 4 | 11 6 | 15 0 | 19 4 | 20 0 | 22 9 |

### EXPERIMENT D2B

To test the permanency of the differences in reproductivity between colonies, four series were set up, 19 weeks after experiment D2 was begun, from four of the colonies used in that experiment. These were designated D2-8B, D2-3B, D2-9B, and D2-8 + 10B in that order. D2-8B consisted of 20 groups, each composed of 5 fifth- and 5 fourth-instar nymphs from the colony which showed the highest reproductivity in experiment D2. Series D2-3B consisted of 9 groups, each composed of 9 sixth-instar nymphs and 1 fifth-instar nymph from the colony from which D2-3 was derived. D2-3, it will be remembered, had by far the lowest reproductivity of the 10 series in experiment D2. Series D2-9B consisted of 11 groups, each composed of 5 fifth- and 5 fourth-instar nymphs from the colony from which series D2-9 was taken, a series among those with low reproductivity.

Series D2-8 + 10B consisted of 20 groups, each composed of 5 fifth-instar nymphs and 5 fourth-instar nymphs. Half of each group was from the colony of origin of D2-8 and half from the colony of origin of D2-10. This was a combination of nymphs,

therefore, from a colony which showed highest reproductivity and one which showed low reproductivity. Table 14 gives the summarized results for these series in terms of (1) per cent of the surviving groups which had produced supplementaries and (2) per cent of original population of surviving groups which had developed into supplementaries by the particular week.

These results show reversal in relative and actual reproductivity. The latter is especially striking in series D2-3B *vs.* D2-3 (fig. 8). Nymphs from colonies which had low reproductivity in experiment D2 (D2-3 and D2-9) here had high reproductivity (table 14), whereas those from the colony which had the highest reproductivity in D2 (D2-8) had here a markedly lower relative reproductivity (8B *vs.* 3B in fig. 8). This reversal is actual in series D2-3 and D2-3B as brought out in figure 8, whereas D2-8B, although it showed much lower reproductivity than D2-3B, agreed closely with the reproductivity recorded for the earlier series D2-8 from the same colony (fig. 8).

Unfortunately for clarity of results, it was necessary to use nymphal types in D2B which were not identical with those used in D2. Older nymphs (sixth instars) were used in D2-3B than in any series of D2, whereas half of the nymphs in the groups of the other three series of D2-B were fourth instars, younger than those used in D2. Since the older nymphs have a higher potential of reproductivity (p. 17), this difference probably had something to do with the very high actual and relative reproductivity of the series D2-3B. That it was entirely responsible seems unlikely because of the marked nature of the reversal and since the presence of younger nymphs in D2-9B, for example, did not prevent it from showing a much higher relative and absolute reproductivity than D2-8B, although D2-8 ranked highest, whereas D2-9 was relatively low (fig. 4).

Since the records fail to state the conditions under which the colonies of origin were kept during the period between experiments D2 and D2B, it is difficult to assign a cause for the supposed change in potential reproductivity. We need to know if the original supplementaries were kept in the colonies and how many other supplementaries had developed during that period. Perhaps, however, the very change in the constitution of the colony resulting from extraction of the nymphs used in experiment D2 explains the change in reproductivity of the nymphs of the colonies from which D2-3B and D2-9B were derived, or perhaps the mere disturbance of the colonies contributed to whatever changes were involved. What seems to be indicated is that the potential reproductivity of a colony is either very impermanent or that it may be readily altered by change in its composition or treatment.

### EXPERIMENT D4A

Another experiment, D4A, carried out considerably earlier, was designed to answer the question of the permanency of the reproductive potential of a colony. Eight series were set up from the same colony at approximately one-month intervals beginning June 21, 1938. Each of the first four series consisted of 20 groups of 10 apterous nymphs of the same early instar, supposedly the fifth. The last four series consisted of 10 such groups. Of these 8 series the second and the last three were discarded because of very high mortality due to epidemics of disease. This left for consideration the four series, D4A-C1, D4A-C3, D4A-C4, D4A-C5. Some groups of these series died out during the experiment and were ignored. There remained 19 groups in each of three series C1, C3, and C4, and 8 of the original 10 groups in C5. In order to present results in comparable terms they are given as percentages of the surviving groups or of the population of the surviving groups.

Table 15 and figure 9 give the essential results. It will be seen that each successive series showed increased reproductivity. That these differences in reproductivity are not due to disease or other differentially unfavorable conditions which would influence the death rate seems indicated by the fact brought out in the last section of table 15, namely, that series D4A-C5 which the remainder of the table will show was persistently highest in reproductivity suffered nevertheless the highest mortality, just the opposite of what would be expected if viability were causally correlated with reproductivity. It is true that D4A-C1, which had the lowest reproductivity, suffered also very high mortality, and here there may well be some causal correlation. Further, since D4A-C3 had much the lowest mortality, the difference in

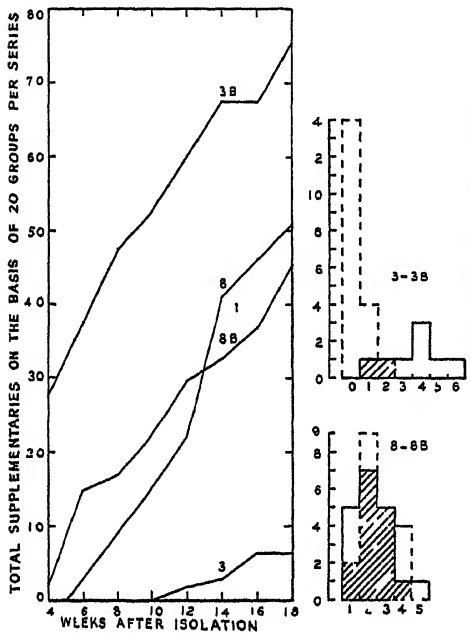


Fig. 8

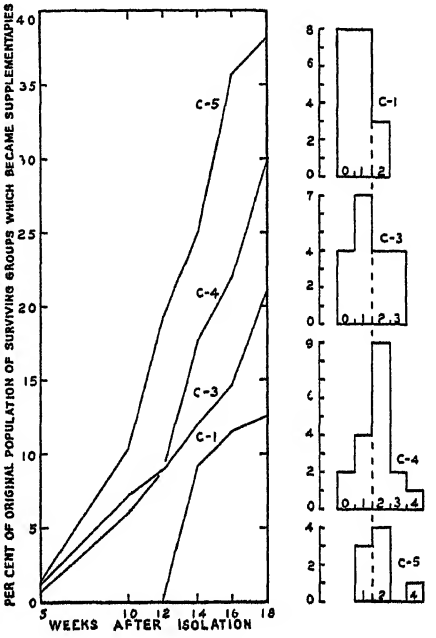


Fig. 9

Fig. 8. Graphs for series 3 and 8 of experiment D2, the series respectively with lowest and highest reproductivity, and for series 3B and 8B of experiment D2B, series from the same colonies as 3 and 8 respectively but taken nineteen weeks later.

Graphs are for total supplementaries by weeks on the basis of 20 groups per series. Groups of the different series although not identical were of similar composition. Note that series 8 and 8B show very similar reproductivity, whereas series 3B shows very much greater reproductivity than did series 3, much greater indeed than either series 8B or 8.

The histograms show frequency distribution of groups by numbers of supplementaries at the eighteenth week for series 3 as contrasted to series 3B, and for series 8B as contrasted to series 8. Series 3 and 8 are represented in broken lines, series 3B and 8B in solid lines, overlapping portions in diagonal lines.

Fig. 9. Experiment D4A. Four series of the same composition taken from the same colony but at different times. Series 1 and 3 are two months apart, 3 and 4, and 4 and 5 one month apart.

Graphs show the percentages of the original population of surviving groups which had become supplementaries at the particular weeks.

Histograms show frequency distribution of existing groups in each series by numbers of supplementaries produced by the eighteenth week. The dotted line between groups with one supplementary and those with two is designed to bring out the shift toward higher reproductivity in successive series. It must be kept in mind that only eight of the ten original groups survived in series 5 and that of the 20 original groups each in series 1, 3, and 4, nineteen survived in each of series 1 and 3, and eighteen in series 4.

reproductivity between series 1 and 3 may well be due largely to differences in condition and not to changes correlated with lapse of time.

The question arises of the significance of these findings. First it seems necessary to point out that until further data are available it cannot be said with certainty that the chronological progress in reproductivity here found is more than a coincidence, nor that the differences between series from the same colony are other than

TABLE 15

REPRODUCTIVITY AND MORTALITY IN SUCCESSIVE SERIES OF THE SAME CONSTITUTION FROM THE SAME COLONY

(Series are listed in chronological order, which is also the order of increasing reproductivity during the later period of the experiment.)

| Group | 5th | 10th | 12th | 14th | 16th | 18th |
|---|---|---|---|---|---|---|
| | Per cent of surviving groups which produced supplementaries (by weeks) | | | | | |
| D4A-C1 | | | | 47 4 | 57 9 | 57 9 |
| D4A-C3 | 10 0 | 47 1 | 57 9 | 78 9 | 78 9 | 78 9 |
| D4A-C4 | 5 0 | 36 7 | 47 4 | 63 2 | 83 3 | 88 9 |
| D4A-C5 | 10 0 | 60 0 | 90 0 | 88 9 | 100 0 | 100 0 |
| | Per cent of original population of surviving groups becoming supplementaries (by weeks) | | | | | |
| D4A-C1 | | | | 5 8 | 6 8 | 7 4 |
| D4A-C3 | 1 0 | 5 3 | 6 3 | 8 4 | 10 0 | 14 2 |
| D4A-C4 | 0 5 | 3 7 | 5 3 | 10 6 | 13 3 | 17 8 |
| D4A-C5 | 1 0 | 7 0 | 11 0 | 13 3 | 18 8 | 20 0 |
| | Per cent of actual population of surviving groups for the given week becoming supplementaries (by weeks) | | | | | |
| D4A-C1 | | | | 9 2 | 11 5 | 12 6 |
| D4A-C3 | 1 2 | 7 2 | 8 0 | 12 0 | 14 6 | 21 1 |
| D4A-C4 | 0 7 | 6 0 | 8 9 | 17 7 | 21 8 | 30 0 |
| D4A-C5 | 1 3 | 10 3 | 19 3 | 25 0 | 35 7 | 38 1 |
| | Per cent of mortality for surviving groups (by weeks) | | | | | |
| D4A-C1 | 19 5 | 30 0 | 32 5 | 37 4 | 40 5 | 41 6 |
| D4A-C3 | 18 0 | 26 8 | 29 5 | 30 0 | 31 1 | 32 6 |
| D4A-C4 | 30 5 | 38 4 | 40 0 | 40 5 | 38 9 | 40 6 |
| D4A-C5 | 23 0 | 32 0 | 43 0 | 46 7 | 47 5 | 47 5 |

those shown to occur by chance between series supposedly of the same origin, composition and treatment, somewhat exaggerated here, perhaps, by the presence of disease and by the effects on the colony itself of the monthly disturbances incidental to setting up these series. It should be kept in mind that in D2-B (p. 24) a reversal in both directions seemed indicated. Further, even if the differences be held to result from changes normally undergone by colonies with the passage of time, there is no information about the nature or causes of these changes. They might represent cyclic seasonal changes in reproductivity undergone by colonies, or a progressive change correlated with the normal development of the colony, or have other unidentified causes.

## DIFFERENCES IN REPRODUCTIVITY BETWEEN SUPPOSEDLY IDENTICAL SERIES RUN CONCURRENTLY

It has been shown earlier in this paper that reproductivity differs (1) between series from different colonies, even though of the same constitution and run concurrently, (2) between series composed of different nymphal types or combinations of such types, although from the same colony and run concurrently, and (3) between series of the same constitution and from the same colony but run at different times.

There remains to be determined the range of differences in reproductivity to be expected between series of identical origin, supposedly identical in composition, subject to the same treatment, and run at the same time. Theoretically, the rate and extent of reproductivity, as also the rate and extent of mortality, should be identical for all the groups and series if all nymphs are from the same colony, if all groups are of the same composition, if they are housed, fed, and handled exactly alike, and are exposed to exactly the same conditions of temperature, moisture, and so on, and if there are no errors of oversight or of decision in making the records.

Actually, of course, there are numerous sources of variation in all these features in experiments involving isolated groups of nymphs (Light, 1942–1943, 1944). Individuals externally similar undoubtedly differ in their position in the intermolt cycle. Nymphs externally alike undoubtedly differ physiologically in this and various other respects, including their degree of sexual inhibition and their relation to the factors, undetermined as yet, which govern development toward alateness, on the one hand, or toward the soldier condition, on the other. Finally, and perhaps most importantly, nymphs of the same type may differ with respect to infection with, or resistance to, the several bacterial and fungous diseases to which they are disposed.

Likewise, as regards treatment, many variables are possible. Containers supposedly alike may vary. Certainly the food varies in quantity, in quality (when wood is used), and also in position in the jar, which may determine its availability. It is inevitable that there should be differences in the duration of exposure to air, in the drying effects of the air, in the amount and type of handling, in the amount of moisture available, and even at times in the temperatures endured.

For one or another or several of these reasons groups within the same series differ markedly, some from the start (see tables 16, 17); and series differ characteristically in the rate and extent of both mortality and reproductivity, the latter whether measured in terms of development of supplementaries, laying of eggs, or appearance of young, or of the totals of each produced per group.

One experiment, 3DS, was run specifically to test the nature and degree of variation in reproductivity and mortality inherent in the materials and methods used. Several other experiments in which there were two or more series of the same constitution and treatment furnish evidence in this regard, although the experiments were designed for other purposes. The latter experiments will be presented first.

### EXPERIMENT LC

LC was designed to test the efficacy of ether extracts of female supplementary reproductives in inhibiting reproductivity. It was partially reported in a previous paper on extract inhibition (1944). Sixty groups of nymphs were used, all from the same colony. Each group consisted of 30 apterous nymphs according to the following formula: 4 of the eighth instar, 9 of the seventh, 6 of the sixth, 8 of the

TABLE 16

DETAILED RECORDS OF THREE IDENTICALLY TREATED EXPERIMENTAL SERIES OF EXPERIMENT LC

| | Group number | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| LC-1 | | | | | | | | | | | | | | | |
| Fifth week | | | | | | | | | | | | | | | |
| Population | 25 | 27 | 24 | 26 | 26 | 28 | 30 | 28 | 24 | 24 | 26 | 24 | 25 | 28 | 26 |
| ♀ Supplementaries | 0 | 0 | 1 | 1 | 0 | 3 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| ♂ Supplementaries | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 |
| Eggs | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Seventh week | | | | | | | | | | | | | | | |
| Population | 24 | 23 | 22 | 25 | 26 | 25 | 28 | 28 | 21 | 22 | 24 | 22 | 23 | 24 | 24 |
| ♀ Supplementaries | 0 | 0 | 1 | 1 | 0 | 3 | 1 | 2 | 1 | 0 | 1 | 1 | 0 | 0 | 1 |
| ♂ Supplementaries | 0 | 2 | 1 | 1 | 0 | 2 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 |
| Eggs | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 24 | 6 | 0 | 4 | 30 | 0 | 0 | 0 |
| Tenth week | | | | | | | | | | | | | | | |
| Population | 22 | 22 | 20 | 25 | 23 | 24 | 28 | 27 | 17 | 19 | 24 | 18 | 23 | 21 | 23 |
| ♀ Supplementaries | 0 | 0 | 1 | 1 | 0 | 3 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 1 |
| ♂ Supplementaries | 0 | 2 | 1 | 1 | 0 | 2 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 |
| Eggs | 0 | 0 | 6 | 2 | 0 | 6 | 12 | 24 | 6 | 0 | 8 | 30 | 0 | 0 | 15 |
| LC-2 | | | | | | | | | | | | | | | |
| Fifth week | | | | | | | | | | | | | | | |
| Population | 28 | 23 | 22 | 21 | 26 | 24 | 27 | 27 | 27 | 22 | 25 | 24 | 22 | 21 | 25 |
| ♀ Supplementaries | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 1 | 1 |
| ♂ Supplementaries | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Eggs | 3 | 0 | 0 | 3 | 3 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 10 | 0 | 2 |
| Seventh week | | | | | | | | | | | | | | | |
| Population | 27 | 22 | 17 | 21 | 25 | 21 | 27 | 27 | 27 | 22 | 24 | 24 | 22 | 20 | 25 |
| ♀ Supplementaries | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 2 | 1 | 1 |
| ♂ Supplementaries | 1 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Eggs | 4 | 0 | 0 | 11 | 10 | 0 | 0 | 11 | 2 | 0 | 0 | 0 | 20 | 0 | 7 |
| Tenth week | | | | | | | | | | | | | | | |
| Population | 26 | 21 | 15 | 19 | 25 | 23 | 24 | 22 | 27 | 19 | 22 | 21 | 21 | 20 | 24 |
| ♀ Supplementaries | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 0 | 3 | 0 | 2 | 1 | 1 |
| ♂ Supplementaries | 1 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| Eggs | 6 | 0 | 1 | 11 | 15 | 0 | 1 | 11 | 14 | 0 | 3 | 0 | 20 | 0 | 10 |
| LC-3 | | | | | | | | | | | | | | | |
| Fifth week | | | | | | | | | | | | | | | |
| Population | 26 | 26 | 20 | 20 | 31 | 24 | 28 | 28 | 21 | 26 | 27 | 28 | 21 | 25 | 27 |
| ♀ Supplementaries | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 3 | 1 | 0 | 1 | 0 | 2 | 2 |
| ♂ Supplementaries | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 0 |
| Eggs | 4 | 1 | 1 | 3 | 10 | 0 | 0 | 1 | 3 | 0 | 0 | 0 | 0 | 1 | 2 |
| Seventh week | | | | | | | | | | | | | | | |
| Population | 26 | 26 | 20 | 27 | 31 | 22 | 25 | 26 | 20 | 23 | 27 | 28 | 21 | 21 | 27 |
| ♀ Supplementaries | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 3 | 1 | 1 | 1 | 0 | 2 | 2 |
| ♂ Supplementaries | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 2 | 0 |
| Eggs | 11 | 10 | 4 | 15 | 20 | 0 | 0 | 2 | 15 | 1 | 2 | 6 | 0 | 10 | 7 |
| Tenth week | | | | | | | | | | | | | | | |
| Population | 25 | 25 | 20 | 16 | 28 | 22 | 24 | 22 | 20 | 22 | 25 | 25 | 14 | 14 | 27 |
| ♀ Supplementaries | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 3 | 2 | 1 | 1 | 1 | 2 | 2 |
| ♂ Supplementaries | 1 | 1 | 1 | 0 | 0 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 0 |
| Eggs | 30 | 15 | 5 | 15 | 24 | 0 | 10 | 11 | 25 | 1 | 15 | 15 | 1 | 10 | 10 |

fifth, and 3 of the fourth and/or third instars. These groups were segregated at random into 4 series of 15 groups each. Three of these series constituted the experimental series and were fed extracts of functional reproductives and treated in supposedly identical fashion. The experiment was continued for 10 weeks, with weekly records from the fifth week. No groups died out, mortality was generally low, about 25 per cent for the 10 weeks, with only a few groups suffering more than

30 per cent mortality, and only one more than 50 per cent. Table 16 gives the detailed results by groups and series for the fifth, seventh, and tenth weeks for the three experimental series which received identical treatment (see also tables 4, 5, and 6, Light, 1944). Figure 10 gives the results for the same three series in graphic form. Reproductivity is expressed in terms of supplementaries produced throughout the experiment. Figure 12, based on the frequency distribution of groups by numbers of supplementaries produced by all four series throughout the experiment, indicates that the differences between series, striking as they seem, are such as to be expected from random variation.

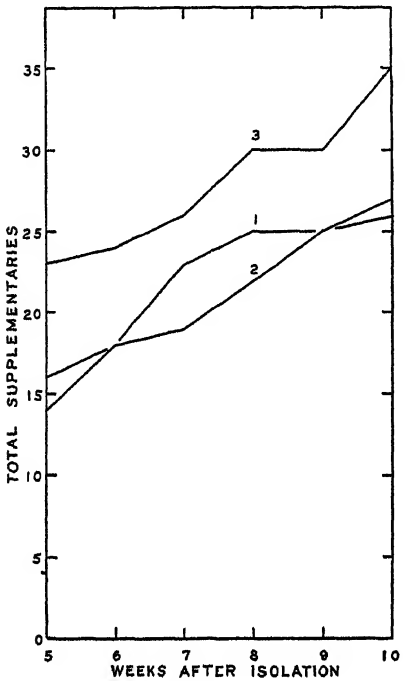


Fig. 10

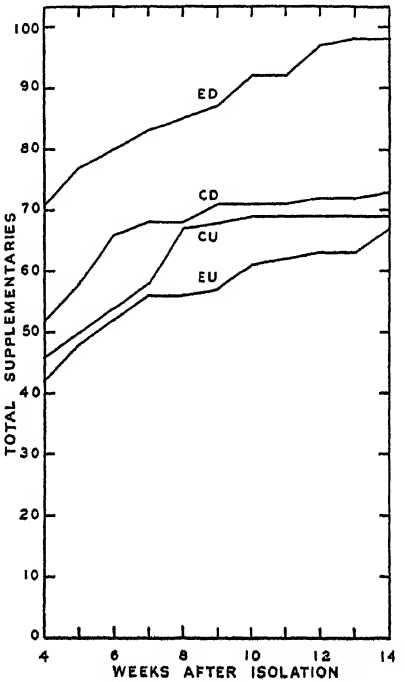


Fig. 11

Fig. 10. Experiment LC. Total supplementaries by weeks for three identical series from the same colony which were given identical treatment and run at the same time.

Fig. 11. Experiment OI: Total supplementaries by series by weeks. Series CD, CN, and EU were identical series from the same colony run at the same time. Series ED consisted of isolated groups from another experiment and had already developed functioning supplementary reproductives.

## EXPERIMENT OI

In experiment OI each of 40 one-ounce jars was separated into two compartments by two perforated mica discs separated by a one-fourth-inch ring of plastic. These two mica discs with the included space served as ceiling for the lower half of the jar and floor of the upper half. Into the lower compartment of half the jars were introduced egg-producing groups from a previous experiment, each containing two or more supplementaries. This series was designated OI-ED. In the upper part of the jars containing series OI-ED, and in both lower and upper compartments of the remaining jars (the controls) were placed groups of 20 nymphs, each group consisting of unpigmented apterous individuals, 6 of the seventh instar, 10

of the sixth instar, and 4 of the fifth instar, all from a single colony which included 3,000 nymphs headed by a primary queen. The series placed in the upper part of the jars containing OI-ED was designated OI-EU, that in the lower part of the control jars OI-CD, and that in the upper part OI-CU. Beginning with the fourth week, weekly records were made of population, numbers of supplementaries, and

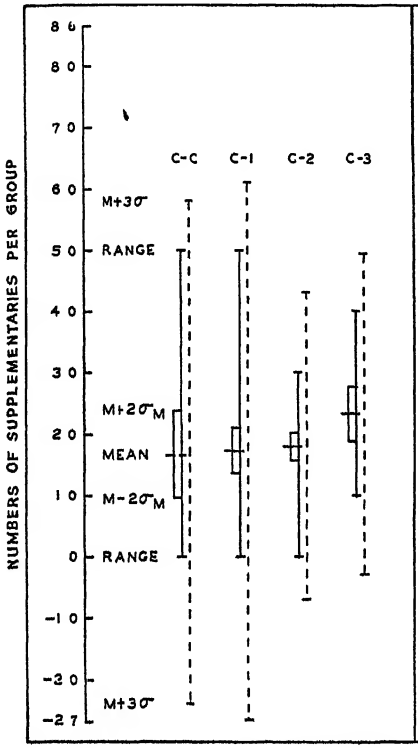


Fig. 12

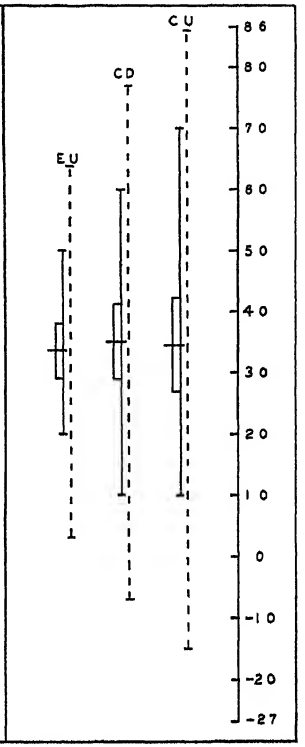


Fig. 13

Fig. 12. Dice-Leraas diagram based on frequency distribution of groups by numbers of supplementaries produced at the tenth week in the four series of experiment LC. All series were of the same composition and origin and run at the same time. C-C was the control series, the other three series were fed extracts of functional reproductives. The close agreement in means and ranges indicates that the differences are due to random incidence of variable factors and give no reason to believe that the extracts had an inhibiting influence. C-3, the series with highest reproductivity, was one of those fed extracts.

Fig. 13. Dice-Leraas diagram based on the frequency distribution of groups by numbers of supplementaries produced by the fourteenth week in the three identical series of experiment OI. Series EU was exposed to odors from functioning supplementaries (see p. 30). Its recorded reproductivity, as brought out in fig. 11, was somewhat less than that of CD and CU which were theoretically not thus exposed. The close agreement in means indicates that the differences between EU and the other two series are not indicative of the effectiveness of odors but results of the random incidence of uncontrolled variables which cause any two such series to differ in recorded reproductivity.

presence of eggs. Figure 11 gives the reproductivity by series in terms of total supplementaries produced. First, it will be noted that the three series of the same constitution and origin (OI-CD, OI-CU, OI-EU) show about the same differences among themselves as in the three series of LC, or the series 3DS, to be discussed later. Put in the form of a Dice-Leraas diagram (fig. 13) based on terminal condi-

tions, there is no indication of significant differences between these three series. Nevertheless, it is of interest to note in passing that the differences observed are in the directions expected. First, the two upper series, OI-EU and OI-CU, had lower reproductivity than OI-CD, which had the highest reproductivity of the three comparable series (fig. 11). This was to be expected, since the bottom of the jar presents a more even and generally more favorable situation. Second, of the two upper series the one over the groups which contained functioning reproductives, OI-EU, showed the lowest reproductivity, which was to be expected if odors given off by functional reproductives inhibit neotenic development of nymphs. However, since the degree of difference is too small to be unquestionably significant, it is not possible to state whether the odor of functional reproductives was responsible for the lower reproductivity in OI-EU, or whether it was due entirely to random incidence of variables in constitution and handling.

Supplementaries and eggs occurred very early in all groups of this experiment. By the fourth week each group in OI-CD contained from one to four supplementaries, and by the sixth week eggs had been recorded from most of its groups. It would appear, therefore, that only at the beginning of the experiment were the two upper series very differently exposed to possibly inhibiting odors from reproductives.

EXPERIMENT 6DS

6DS was reported earlier in this paper in connection with differences in reproductivity between series from the same colony run at the same time and given the same treatment but of different constitution as regards types of nymphs (see tables 7–10 and figs. 2 and 3). Each of the series consisted of 40 groups. Figure 2 presents by means of graphs the reproductivity by series of 40 groups on the basis of number of supplementaries produced. It is of interest here to consider each of these large series as divided arbitrarily into two series of 20 groups each in order to get some picture of the extent of the range of random difference. Figure 3 presents graphically reproductivity for the eight subseries resulting when the four homogeneous series of 6DS are thus subdivided. The differences in reproductivity between subseries consisting of groups of nymphs of the same type, although definite, is clearly much less in most instances than that between series each made up of groups of nymphs of a different type. The greatest differences between subseries were found in the series of broad-headed nymphs, especially those made up of apterous broad-heads. This may be the result of unconscious selection in making up these groups. Perhaps the most distinctive individuals of the type (not ordinarily abundant) were used up in the first half of the series and those used in the latter half included preponderantly those of earlier instars and even perhaps some not actually belonging to this category.

EXPERIMENT 3DS

3DS was set up from a single colony of *Zootermopsis nevadensis* in January, 1941. This colony was without supplementaries and was headed by a primary physogastric queen. The king was presumably lost in removing the colony from the log. The population included 2,567 nymphs (18.5 per cent wing-padded, 16.5 per cent broad-headed apterous, 20 per cent apterous seventh instar, 15.5 per cent apterous sixth instar, 34 per cent fourths and thirds). Very young nymphs, and soldiers were not recorded.

Each experimental group was so constituted as to approximate relations in the natural colony, and consisted of 3 wing-padded nymphs (chiefly sixth instars), 3 broad-headed apterous nymphs (presumably eighth instar), 4 apterous nymphs of

the seventh instar, 4 of the sixth, 5 of the fifth, and 1 of the fourth. Observations were made weekly for all series except C4. The latter was not handled until the fourth week in an effort to determine whether early handling induced high mortality. This series showed, however, the highest mortality (fig. 15), a situation to be explained probably in terms of humidity and consequent growth of microorganisms rather than handling. During the four weeks when the groups and containers of this series were not subjected to drying out during handling, microorganisms were presumably able to grow unchecked. It should be noted, however, that in spite of high mortality series C was among the highest in reproductivity toward the end of the experiment (fig. 14).

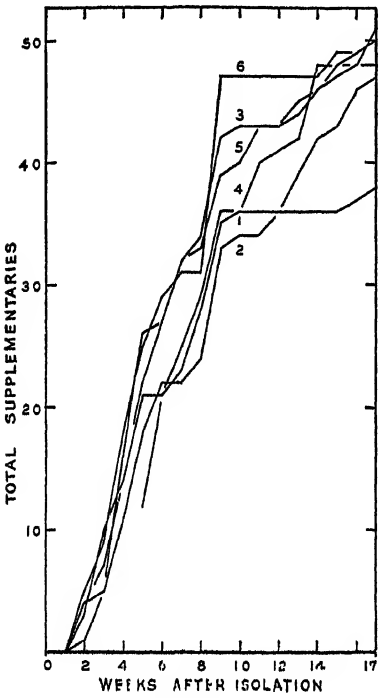


Fig. 14

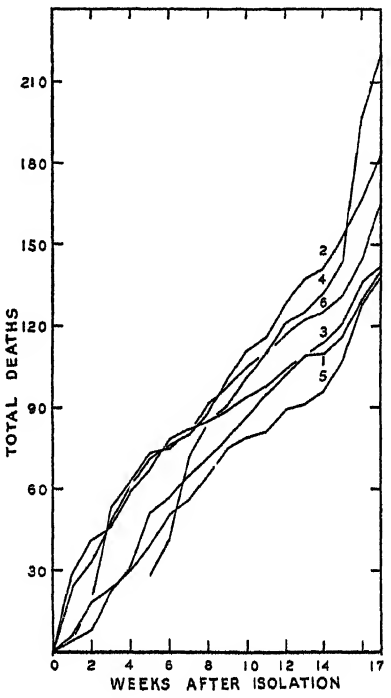


Fig. 15

Fig. 14. Total supplementaries by weeks for the six series of experiment 3DS, each consisting of twenty groups of nymphs of the same composition, from the same colony, run at the same time and given the same treatment with the exception of C-4 as explained in the text. No correction has been made for groups which died out during the experiment.

Fig. 15. Mortality by weeks for the six series of 3DS. Groups which died out during the experiment are included in deaths.

For each group of each series weekly records were kept of the number of living individuals (population), of the number of supplementaries present as recognized by pigmentation, and of the presence of eggs. Table 17 gives the detailed raw data for one series, 3DS-C1, adjusted to express maximum extent of production of supplementaries by continuing in the weekly totals of supplementaries the supplementaries known to have died (indicated by figures preceded by plus sign). Figure 14 shows by means of graphs the total supplementaries known to have been produced by each series at each recording; figure 15 gives mortality for each series for each recording including groups which died out during the experiment; and figure

## TABLE 17

### Detailed Raw Data for Series 3DS-C1

(Corrected only for supplementaries known to have died during the experiment or assumed to have died; see p. 33.)

| | Group number | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| **First week** | | | | | | | | | | | | | | | | | | | | |
| Population | 20 | 20 | 20 | 19 | 20 | 20 | 20 | 20 | 19 | 20 | 20 | 20 | 20 | 20 | 20 | 19 | 20 | 20 | 19 | 20 |
| **Second week** | | | | | | | | | | | | | | | | | | | | |
| Population | 20 | 20 | 20 | 19 | 20 | 20 | 20 | 19 | 19 | 19 | 19 | 20 | 20 | 20 | 19 | 19 | 20 | 20 | 19 | 20 |
| Supplementaries | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **Third week** | | | | | | | | | | | | | | | | | | | | |
| Population | 20 | 18 | 20 | 19 | 20 | 19 | 19 | 18 | 18 | 18 | 18 | 20 | 20 | 20 | 19 | 18 | 20 | 15 | 19 | 20 |
| Supplementaries | 0 | 0 | 0 | 0 | 1 | 1 | $0^{+1}$ | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| **Fourth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 20 | 18 | 19 | 19 | 20 | 17 | 19 | 17 | 18 | 18 | 18 | 20 | 20 | 18 | 19 | 18 | 20 | 12 | 19 | 20 |
| Supplementaries | 0 | 1 | 0 | 0 | 1 | 1 | $1^{+1}$ | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| **Fifth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 19 | 18 | 19 | 19 | 19 | 17 | 19 | 17 | 18 | 18 | 17 | 19 | 20 | 18 | 18 | 18 | 18 | d[a] | 18 | 20 |
| Supplementaries | 0 | 1 | 1 | 1 | 1 | 1 | $1^{+1}$ | 1 | 0 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 0 | | 1 | 1 |
| Eggs | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 |
| **Sixth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 18 | 18 | 19 | 18 | 19 | 16 | 19 | 17 | 18 | 18 | 17 | 19 | 20 | 18 | 16 | 18 | 18 | | 17 | 20 |
| Supplementaries | 0 | 1 | 1 | 1 | 1 | 1 | $1^{+1}$ | 2 | 0 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 0 | | 1 | 1 |
| Eggs | 0 | 0 | + | 0 | + | + | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | | 0 | 0 |
| **Seventh week** | | | | | | | | | | | | | | | | | | | | |
| Population | 18 | 18 | 19 | 18 | 17 | 15 | 19 | 17 | 18 | 18 | 17 | 19 | 20 | 18 | 14 | 17 | 15 | | 15 | 20 |
| Supplementaries | 0 | 1 | 1 | 1 | 1 | 1 | $1^{+1}$ | 2 | 0 | 1 | 2 | 1 | 2 | 1 | $1^{-1}$ | 1 | 0 | | 1 | 2 |
| Eggs | 0 | 0 | + | 0 | + | + | + | + | 0 | 0 | 0 | 0 | + | + | 0 | 0 | 0 | | + | + |
| **Eighth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 18 | 18 | 19 | 18 | 16 | 15 | 17 | 17 | 18 | 17 | 17 | 19 | 19 | 18 | 14 | 17 | 18 | | 14 | 20 |
| Supplementaries | 0 | 1 | 1 | 1 | 2 | 2 | $2^{-1}$ | 2 | 1 | 1 | 2 | 1 | 2 | 1 | $1^{-1}$ | 1 | 0 | | 2 | 2 |
| Eggs | 0 | 0 | + | 0 | + | + | + | + | 0 | 0 | 0 | 0 | + | + | 0 | + | 0 | | + | + |
| **Ninth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 18 | 17 | 19 | 18 | 15 | 15 | 17 | 16 | 18 | 16 | 17 | 19 | 18 | 16 | 14 | 16 | 18 | | 14 | 20 |
| Supplementaries | 0 | $0^{-1}$ | 2 | 2 | 2 | 2 | $2^{+1}$ | 2 | 1 | 1 | 2 | 3 | 3 | 1 | $1^{-1}$ | 2 | 1 | | 2 | 2 |
| Eggs | 0 | 0 | + | 0 | + | + | + | + | 0 | 0 | 0 | 0 | + | + | 0 | + | 0 | | + | + |

[a] Group 18 extinct from the fifth week.

| | Group number | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| **Tenth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 18 | 16 | 19 | 18 | 15 | 14 | 17 | 16 | 18 | 14 | 17 | 18 | 17 | 15 | 14 | 16 | 18 | | 14 | 19 |
| Supplementaries | 0 | $0^{-1}$ | 2 | 2 | 2 | $1^{-1}$ | $2^{+1}$ | 2 | 1 | 1 | 2 | $2^{-1}$ | 3 | 1 | $1^{-1}$ | 2 | 2 | | 2 | 2 |
| Eggs | 0 | 0 | + | 0 | + | + | + | + | 0 | 0 | 0 | 0 | + | + | 0 | + | 0 | | + | + |
| **Eleventh week** | | | | | | | | | | | | | | | | | | | | |
| Population | 18 | 15 | 19 | 18 | 14 | 14 | 17 | 16 | 17 | 13 | 17 | 17 | 16 | 15 | 14 | 15 | 17 | | 14 | 19 |
| Supplementaries | 0 | $0^{-1}$ | 2 | 2 | 2 | $1^{+1}$ | $2^{-1}$ | 2 | 1 | $0^{+1}$ | 2 | $2^{+1}$ | 3 | 1 | $1^{+1}$ | 2 | 2 | | 2 | 2 |
| Eggs | 0 | 0 | + | 0 | + | + | + | + | 0 | 0 | 0 | 0 | + | + | 0 | + | + | | + | + |
| **Twelfth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 17 | 15 | 18 | 17 | 14 | 14 | 17 | 16 | 17 | 13 | 17 | 16 | 16 | 15 | 14 | 13 | 16 | | 14 | 19 |
| Supplementaries | 0 | $0^{-1}$ | 2 | 2 | 2 | $1^{+1}$ | $2^{-1}$ | 2 | 1 | $0^{+1}$ | 2 | $2^{+1}$ | 3 | 1 | $1^{+1}$ | 2 | 2 | | 2 | 2 |
| Eggs | 0 | 0 | + | + | + | + | + | + | 0 | 0 | 0 | 0 | + | + | 0 | + | + | | + | + |
| **Thirteenth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 16 | 15 | 18 | 17 | 14 | 13 | 17 | 16 | 16 | 12 | 15 | 16 | 16 | 15 | 14 | 13 | 16 | | 14 | 18 |
| Supplementaries | 0 | $0^{-1}$ | 2 | 2 | 2 | $1^{+1}$ | $2^{+1}$ | 2 | 1 | $0^{+1}$ | 2 | $2^{+1}$ | 3 | 1 | $1^{-1}$ | 2 | 2 | | 2 | 2 |
| Eggs | 0 | 0 | + | + | + | + | + | + | 0 | 0 | 0 | + | + | + | + | + | + | | + | + |
| **Fourteenth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 16 | 15 | 18 | 17 | 13 | 13 | 17 | 16 | 16 | 12 | 15 | 16 | 16 | 15 | 14 | 13 | 16 | | 14 | 18 |
| Supplementaries | 0 | $0^{+1}$ | 2 | 2 | 2 | $1^{+1}$ | $2^{+1}$ | 2 | 1 | $0^{-1}$ | 2 | $2^{+1}$ | 3 | 1 | $1^{+1}$ | 2 | 2 | | 2 | 2 |
| Eggs | 0 | 0 | + | + | + | + | + | + | 0 | 0 | 0 | + | + | + | + | + | + | | + | + |
| **Fifteenth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 16 | 15 | 18 | 17 | 10 | 13 | 16 | 16 | 16 | 11 | 14 | 16 | 16 | 15 | 14 | 13 | 16 | | 14 | 18 |
| Supplementaries | 0 | $0^{+1}$ | 2 | 2 | 2 | $1^{+1}$ | $1^{+2}$ | 2 | 1 | $0^{+1}$ | 2 | $2^{-1}$ | 3 | 1 | $1^{+1}$ | 2 | 2 | | 2 | 2 |
| Eggs | 0 | 0 | + | + | + | + | + | + | 0 | 0 | 0 | 0 | + | + | + | + | + | | + | + |
| **Sixteenth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 16 | 11 | 17 | 16 | 9 | 12 | 15 | 16 | 15 | 10 | 13 | 16 | 16 | 14 | 14 | 12 | 16 | | 14 | 18 |
| Supplementaries | 0 | $0^{+1}$ | 2 | 2 | $1^{+1}$ | $1^{+1}$ | $1^{+2}$ | 2 | 1 | $0^{+1}$ | 2 | $3^{+1}$ | 3 | $0^{+1}$ | $1^{+1}$ | 2 | 2 | | 2 | 2 |
| Eggs | 0 | 0 | + | + | + | + | + | + | 0 | 0 | 0 | + | + | + | + | + | + | | + | + |
| **Seventeenth week** | | | | | | | | | | | | | | | | | | | | |
| Population | 15 | 10 | 15 | 16 | 9 | 12 | 14 | 16 | 15 | 10 | 10 | 16 | 16 | 14 | 14 | 10 | 15 | | 14 | 18 |
| Supplementaries | 0 | $0^{+1}$ | 3 | 2 | $1^{+1}$ | $1^{+1}$ | $1^{+2}$ | 2 | 1 | $0^{+1}$ | 2 | $3^{+1}$ | 3 | $0^{+1}$ | $1^{+1}$ | 2 | $1^{-1}$ | | 2 | 2 |
| Eggs | 0 | 0 | + | + | + | + | + | + | + | 0 | 0 | + | + | + | + | + | + | | + | + |
| **Total supplementaries** | 0 | 1 | 3 | 2 | 2 | 2 | 3 | 2 | 1 | 1 | 2 | 4 | 3 | 1 | 2 | 2 | 2 | | 2 | 2 |

16B shows the homogeneity of the 6 identical series of 3DS from the same colony by spread of frequency of numbers of supplementaries per group. The contrasting heterogeneity of 5 identical series from different colonies (4DS) is shown in figure 16A.

The records of these series, as of all other such experiments with isolated groups of nymphs, show great differences between individual groups in reproductivity (table 17) and even greater differences in mortality. Group 1, for example, pro-

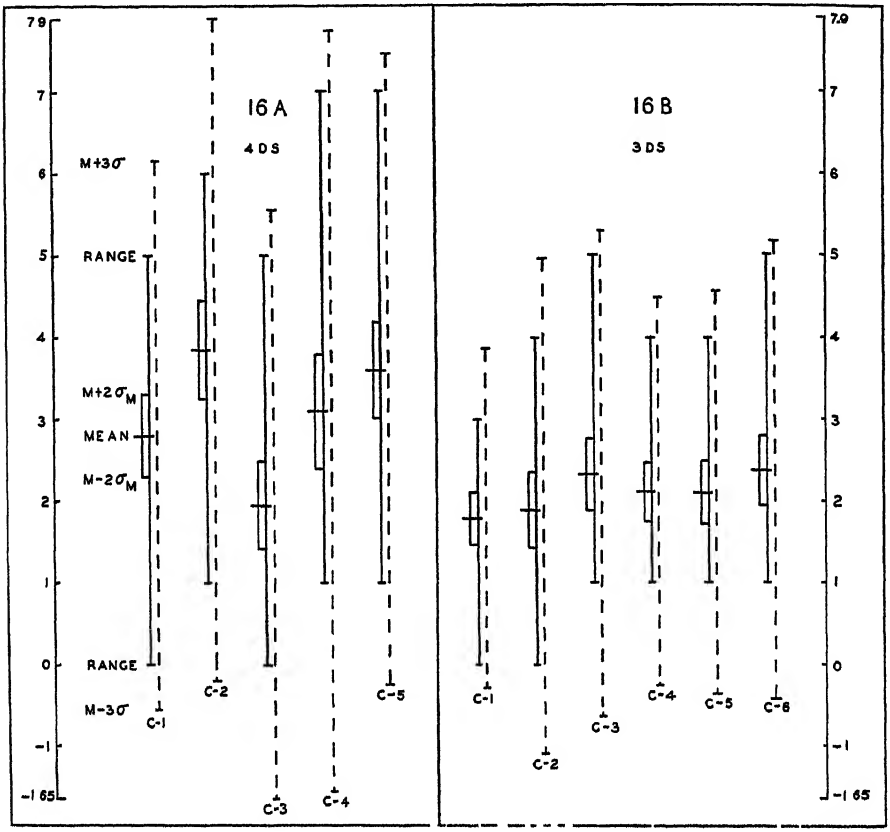


Fig. 16, *a*. Dice-Leraas diagram based on the frequency distribution of groups by numbers of supplementaries produced at the twelfth week for the six series of experiment 4DS, each series from a different colony. The spread in location of the means and ranges, theoretical and observed, indicates that the samples are not from the same population.

Fig. 16, *b*. Dice-Leraas diagram, derived as in figure 16, *b*, for the six series of experiment 3DS, all series being identical in composition and from the same colony. The essential homogeneity of the results is that to be expected from samples of the same population. The differences between series presumably arise here as in OI (fig. 13) from the random incidence of uncontrolled variables.

duced no supplementaries, whereas Group 12 produced 4, and three groups produced 3 each, ten produced 2 each, and four produced 1 each. It will be seen from table 17 that all groups of series 1 suffered some mortality, and this is true of practically all of the groups of nymphs used in our many experiments. In spite of all efforts to improve conditions and handling, mortality persists. Its causes are not entirely clear (see p. 3). That it is due in part to cannibalism seems probable.

That it is normal in the life of the colony seems also probable. There is some evidence that it functions in isolated groups to enable the group to arrive at the necessary physiological adjustment. For example, mortality has been found in the instances studied to be higher in groups of 20 than in groups of 10, whereas the general condition of the group seems better in groups of 20.

Mortality as such, if uniform or relatively so and due to factors equally distributed in all groups, would be annoying but would not cloud the issues of the experiment. Much of the mortality, however, is distinctly differential, affecting certain groups far more than others, and seems to be due to the incidence of contagious disease. Some groups are wiped out, some early in the experiment (as Group 18 in 3DS-C1, table 17), some later. Other groups, although obviously infected, survive as groups but with greatly lowered population (as Group 5, table 17).

Although it is common to lose one or more groups during an experiment, some experiments such as 4DS, for instance, suffer no loss of groups and show a relatively low mortality (table 12). In 4DS at the seventeenth week only one group out of 100 had lost more than 50 per cent by death. In 61 groups 75 per cent or more of the original population survived the experiment, 12 groups lost only one individual each, and one group retained its original population.

Differential mortality between groups and series, therefore, is a complicating factor to be taken into account in evaluating the significance of differences in reproductivity. A strong negative correlation between mortality and reproductivity would be expected if mortality is due to disease or unfavorable environmental circumstances, since these would be expected to slow down or prevent normal physiological processes. When striking instances are considered, such a correlation is obvious for the individual group. When all groups of experiment 3DS are arbitrarily segregated into two series, those with less than 13 individuals at the seventeenth week and those with 13 or more, the average number of supplementaries produced by the groups with low population is 1.44 supplementaries per group, whereas it is 2.15 per group for those with higher population. Similarly the average population is found to be considerably greater for those groups which produced eggs than for those which did not.

As brought out in figures 14 and 15, the series taken as wholes differ in reproductivity, however measured, as also in mortality. The differences in reproductivity between like series might naturally be thought to be explained by differences in mortality of the constituent groups, but a comparison of the graphs showing total numbers of supplementaries (fig. 14) with those for mortality (fig. 15) shows no significant correlation. Series 3DS-C1 with very low mortality has the lowest reproductivity instead of the highest, as would be expected if mortality were the significant factor in determining these differences in reproductivity. Further, a scatter diagram for all groups of the experiment shows no correlation between mortality and reproductivity.

## SUMMARY

Earlier work has shown that although all nymphs above the third instar are capable of becoming sexually mature while retaining most of the nymphal characters of the instar in which they were isolated, not all nymphs in isolated groups do become neotenic reproductives (supplementaries). The failure of some nymphs in isolated groups to become supplementaries has been shown to be due to the presence of the supplementary reproductives which do develop, since when the latter are removed a further number of nymphs become supplementaries. In other words, the extent to which supplementaries develop is influenced by the composition of the group.

1. In the study here reported it has been found that reproductivity is most effectively measured in terms of the number of supplementaries produced in a given group or series of groups within a given time after isolation from the parent colony. Other criteria which are in general agreement with the above criterion are: numbers of groups in which supplementaries develop, numbers of groups in which eggs or young are produced, and the number of eggs or young produced.

2. Series from different colonies showed marked differences in the rate and extent to which supplementaries developed even though the series were run concurrently and the groups were the same as to types and numbers of nymphs.

3. Series from the same colony, run at the same time but composed either of different nymphal types or of different combinations of types, showed marked differences in reproductivity. In general, when the groups were composed entirely of one instar, the younger instars showed low reproductivity, apterous nymphs had higher reproductivity than did brachypterous (wing-padded) nymphs, and highest of all were the nymphs of very late instars, the so-called broad-heads, which are usually apterous but may possess wing pads. However, our experience with large groups of mixed composition although not documented by actual experiments, indicates that in such groups the supplementaries are often of a comparatively young instar.

4. Series of groups of the same or similar composition from the same colony but run at successive intervals showed great differences, which indicates either that the potential reproductivity is very unstable, readily altered by handling and change of composition, or that it is subject to relatively rapid change, possibly cyclic in nature.

In one such experiment (D4A) each successive series showed an increase in reproductivity. In another (D2-B), there was a reversal of relative reproductivity; the colony which had the lowest reproductivity in the original experiment later showed high reproductivity.

5. A considerable degree of variation in reproductivity has been found to obtain between series of the same constitution, origin, and treatment, when run concurrently. Statistical methods show these differences to fall within the range to be expected from random variation.

6. The differences pointed out above between series of different origin, different composition, or taken at different times are greater than those which are due to random differences and would seem to be significant, although further evidence is required before these conclusions can be considered fully established.

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# A NEW RECESSIVE LETHAL MUTATION IN MICE

BY

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# A NEW RECESSIVE LETHAL MUTATION IN MICE

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## INTRODUCTION

THE PURPOSE of this paper is to present the genetic, experimental, and pathological data obtained in the investigation of a new mutation in the house mouse, *Mus musculus*. The affected animals were characterized by muscular incoördination and tetany leading to death. The name jittery was tentatively applied to the mutant during the course of the experiments and will be retained until the physiological cause is definitely established. The symbol "j" will be used to represent the recessive gene for jittery.

In view of the widespread occurrence of related types of anomalies in other animals, and in man, it is important to present facts that may be pertinent to the whole group of diseases. With this thought in mind, investigations were undertaken to find the physiological and anatomical basis of this mutant character

The author is indebted to Dr. S. J. Holmes for friendly counsel and to Mr. C. J. Olberg for excellent care of the animals used in these experiments.

## DESCRIPTION OF THE MUTANT

Animals which became jittery could not be distinguished from their normal litter mates until they were from 10 to 16 days old. Muscular incoördination was the first symptom noticed. This was most easily demonstrated by forcing the young mice to run about. The affected ones followed an irregular, zigzag course in attempting to maintain an upright position while running. When placed on their backs, the normal mice righted themselves immediately, whereas the affected animals went through a series of random movements before they succeeded. The mean age at onset of symptoms was $12.11 \pm 0.463$ days.

Within 48 hours after onset, the lack of muscular coördination became so marked that the animals were unable to run for any distance without falling; in severe cases, forward movement was accomplished by creeping. When at rest, they assumed a squatting position, so that they appeared to crouch on their heels. Normal and affected animals are shown in plate 1, figures 1 and 2.

Tetany appeared at about 72 to 96 hours after the onset of symptoms. At first, only the forelegs were affected during periods of excitement or great muscular effort. During the attacks, the hind legs were spread out more than usual, the muscles of the whole body appeared taut, the head was thrust forward and downward, and the forelegs were extended forward under the throat. The forelegs, which were affected most by the tetany, beat up and down rapidly for a period of 2 to 3 seconds. After the spasms, the animals relaxed and appeared exhausted. Their muscles being spastic, forward motion was accomplished either by lurches, accompanied by many random movements usually ending in tetany and a complete loss of equilibrium, or by a slow, cautious, creeping movement. One striking peculiarity was the animals' use of their tails and noses in regaining an upright position. When they fell on their sides, they arched their backs so that their noses and tails supported most of their weight.

During the fourth week of life, the condition became more pronounced and the animals moved very little. Their coats became rough, partly because they were unable to groom themselves, and probably also because they were unable to obtain sufficient nourishment. The difference in size between affected and normal animals became very noticeable; the former lost weight daily and, therefore, appeared emaciated. Tetany became more frequent until the day before death, when the animals appeared too weak to move. The mean age at death of 29 affected animals under daily observation was 31.89 ± 0.55 days.

## ORIGIN OF THE MUTANT

On April 27, 1936, a litter of one male and six female mice was born from a $P_1$ mating of Bagg albino ♂ 6 × Bagg albino ♀ 8. Six of these failed to grow normally and showed a peculiar type of muscular incoördination, with frequent attacks of

TABLE 1

HISTORY OF THE BAGG ALBINO STOCK AT COLD SPRING HARBOR FROM WHICH JITTERY AROSE

| | Generations | | | | |
|---|---|---|---|---|---|
| 34 | 35 | 36 | 37 | 38 | 39 |
| ♂ 30879 | ♂ 35059 | ♂ 44268 | ♂ 52047–♂ 6*<br>♀ 52048–♀ 7<br>♀ 52049–♀ 8*<br>♀ 52050–♀ 9*<br>♀ 52051–♀ 10* | | |
| | ♂ 35060 | ♂ 37027 | ♂ 40732 | ♂ 48085 | ♂ 52489–♂ 1<br>♀ 52590–♀ 2<br>♀ 52591–♀ 3<br>♀ 52592–♀ 4<br>♀ 52593–♀ 5 |

* Heterozygous $J_1$ animals.

tetany. They became emaciated and died within a month. The remaining normal animal was a female, and, when backcrossed to her father (♂ 6), produced a litter of ten, three of which died of the same peculiar trait. In the meantime, a mating of ♂ 6 × ♀ 9 has produced four young, three of which died under similar circumstances. Later, one of the ten $F_1$ progeny of a $P_1$ cross of ♂ 6 × ♀ 10 died under the same circumstances. Female 7, when mated to ♂ 6, produced only normal offspring.

It appeared that ♂ 6 and at least three of his female sibs carried recessive lethal genes for this trait. Male 6 and his siblings ♀ 7, ♀ 8, ♀ 9, and ♀ 10 were obtained from the Carnegie Institution of Washington through the courtesy of Dr. E. C. MacDowell. The shipment included another sibship, namely, Bagg albino ♂ 1 and his sibs, ♀ 2, ♀ 3, ♀ 4, and ♀ 5, but these did not produce affected offspring when mated among themselves or to ♂ 6 and his sibs. Since ♂ 6 and three of his female sibs produced the trait in their offspring but did not show it themselves, it is probable that a recessive gene for the trait arose some generations before in the colony at the Carnegie Institution. Dr. MacDowell states (personal communication) that no such trait has appeared in his colony. A copy of the record card supplied by Dr. MacDowell is reproduced in table 1, showing that ♂ 1 and his female sibs arose from the same inbred line as ♂ 6 and his female sibs, but at an earlier date.

It is impossible to tell when the mutation appeared, but since the inbred line from which ♂ 1 arose had common ancestors in the line from which ♂ 6 arose and, further, since no affected animals appeared in the ancestry of ♂ 1 after it was separated from the main strain, it seems unlikely that the mutation occurred before the 35th generation.

Systematic breeding experiments were undertaken to increase the stock and establish the exact method of transmission of the trait.

## REVIEW OF THE LITERATURE

The literature contains descriptions of a number of conditions which resemble jittery in certain details. Dana (1887) presented a human family pedigree of hereditary tremors which he attributed to a dominant gene. Batten and Wilkinson (1914) reviewed the literature on a type of human ataxia, originally described by Merzbacher (1910), and concluded that the malady was inherited as a sex-linked recessive trait. Bergman (1920) presented some fifty cases of a mild form of human hereditary tremors which he believed to be due to a dominant gene.

Cases of hereditary muscle contracture among the new-born have been reported in cattle by Hutt (1934), in swine by Hallquist (1933), and in sheep by Roberts (1926, 1929). In each of these a recessive lethal gene was involved. Stockard (1936) recorded a case of hereditary posterior paralysis in crosses between St. Bernard and Great Dane dogs. Three dominant genes were involved, two of them carried by one breed and one by the other.

A single recessive gene producing ataxia in pigeons was described by Riddle (1917). Hutt (1932) and Hutt and Child (1934) reported a semilethal mutation in chicks which caused muscular incoördination of the neck and legs.

The congenital palsy in guinea pigs, reported by Cole and Ibsen (1920), resembles jittery in several respects. The young guinea pigs were of normal weight at birth, but showed tremors and tetany. They were unable to stand, and responded to auditory stimuli by falling on their sides in tetanic spasms. Most of them died within 14 days without showing much gain in weight. The malady was due to a recessive lethal gene. A similar condition in *Peromyscus* was described by Huestis and Barto (1936). The animals were normal at birth, but began to show tremors during the third week of life, and these increased in severity until death at about 35 days of age. Except for the absence of tetany, this condition resembled jittery very closely. It was due to a recessive lethal gene.

## GROWTH OF THE JITTERY ANIMALS

In the animals homozygous for jittery, the typical symptoms began to appear about two weeks after birth. Table 2 shows the mean age at onset of the malady in each of three groups kept under daily observation. The first group consisted of 25 progeny from heterozygous Bagg albino matings. The mean age at which the affected individuals in this group could be recognized was $12.64 \pm 0.89$ days. The remaining two groups were made up of backcross progeny obtained by outcrossing the heterozygous Bagg albino males to normal dba and C57Blk females, and then backcrossing their $F_1$ progeny to heterozygous Bagg albinos.

Among the dba backcross group, only six affected animals were considered. Their mean age at onset was $11.16 \pm 0.11$ days. The C57Blk backcross group contained sixteen jittery animals with a mean age at onset of $12.53 \pm 0.58$ days. The standard deviation of the mean age at onset in the Bagg albino group was 4.46 days. It was much larger than those of the other two groups, owing to the appearance of several

jittery animals at an early age. One case was recognized on the fourth day after birth, and two others on the fifth day.

Text figures 1, 2, and 3 show the growth curves of the three groups of jittery animals and their normal siblings. The litters were observed daily and weighed every

TABLE 2

AGE AT ONSET OF SYMPTOMS AND AT DEATH OF JITTERY MICE FROM VARIOUS MATINGS

| Mating | Age at onset | | | Age at death | | |
|---|---|---|---|---|---|---|
| | No. | Mean | Stand. dev. | No. | Mean | Stand. dev. |
| Bagg albino Jj × Jj..... | 25 | 12.64±0.89 | 4.46 | 15 | 31.93±0.83 | 3.172 |
| dba backcross Jj × Jj..... | 6 | 11.16±0.11 | 0.282 | .. | ..... | ..... |
| C57Blk backcross Jj × Jj.... | 16 | 12.53±0.58 | 1.130 | 14 | 31.85±0.80 | 2.799 |
| Total..... | 44 | 12.11±0.46 | 3.035 | 29 | 31.89±0.55 | 3.009 |

TABLE 3

MEAN WEIGHTS (IN GRAMS) OF NORMAL AND JITTERY MICE FROM THREE TYPES OF MATINGS

| Age in days | Bagg albino × Bagg albino | | Bagg albino × $F_1$ (Bagg albino × dba) | | Bagg albino × $F_1$ (Bagg albino × C57Blk) | |
|---|---|---|---|---|---|---|
| | Normal | Jittery | Normal | Jittery | Normal | Jittery |
| 1..... | 1.43 | 1.44 | 1.35 | 1.49 | 1.44 | 1.39 |
| 3..... | 2.24 | 2.22 | 2.18 | 2.60 | 2.04 | 1.98 |
| 5..... | 3.24 | 3.16 | 2.85 | 3.10 | 2.97 | 2.90 |
| 7..... | 4.05 | 3.99 | 3.77 | 4.00 | 4.22 | 3.92 |
| 9..... | 4.93 | 4.95 | 4.84 | 4.91 | 5.20 | 4.80 |
| 11..... | 6.13 | 5.92 | 5.76 | 6.03 | 6.11 | 5.37 |
| 13..... | 6.77 | 6.48 | 6.47 | 6.62 | 6.67 | 6.01 |
| 15..... | 7.33 | 7.00 | 6.85 | 6.99 | 7.27 | 6.41 |
| 17..... | 7.55 | 6.95 | 7.18 | 6.83 | 7.85 | 6.43 |
| 19..... | 8.13 | 6.75 | 7.69 | 6.42 | 8.43 | 6.53 |
| 21..... | 8.80 | 6.75 | 8.19 | 6.06 | 9.19 | 6.53 |
| 23..... | 10.08 | 6.48 | 9.18 | 5.94 | 10.09 | 6.26 |
| 25..... | 10.08 | 6.22 | 9.96 | 5.87 | 10.92 | 6.75 |
| 27..... | 11.40 | 6.21 | 10.80 | 5.67 | 12.53 | 7.17 |
| 29..... | 12.73 | 5.73 | 11.66 | 6.18 | 13.61 | 6.71 |
| 31..... | 14.38 | 5.71 | 13.33 | 5.98 | 14.87 | 6.58 |
| 33..... | 15.04 | 6.01 | 15.77 | 5.02 | 16.35 | 5.59 |
| 35..... | 14.86 | 5.60 | ..... | .... | 17.78 | 6.66 |

second day. The first day shown on the charts was the first day after birth. Data from which the growth curves were made are shown in table 3.

The first significant difference in weight between the affected and the normal animals appeared on the ninth day among the backcross offspring of the heterozygous Bagg albino × normal C57Blk matings. Among the Bagg albinos and the heterozygous Bagg albino × dba backcross groups, a significant difference in weight appeared on the seventeenth day. The number of jittery animals in the dba backcross group was too small to be reliable and, therefore, could not be compared with the

C57Blk group. The number of affected mice of the Bagg albino group was large enough for comparison and showed a significant difference between the two groups after the ninth day.

The reason for the slow growth of the jittery animals in the backcross group is not evident from the data presented here, but it may have been due to modifying

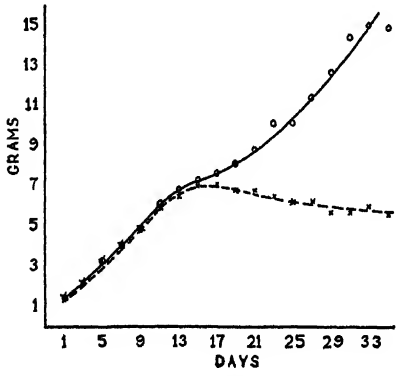


Fig. 1. Curves showing the growth of normal (solid line) and jittery (broken line) $F_1$ progeny from Jj Bagg albino × Jj Bagg albino matings.

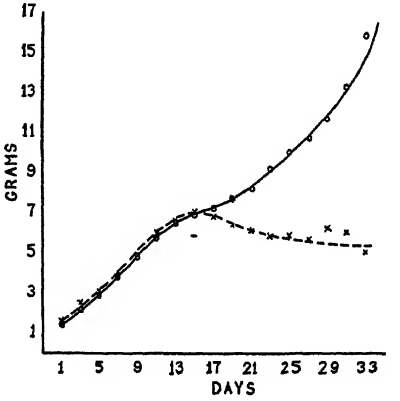


Fig. 2. Curves showing the growth of normal (solid line) and jittery (broken line) backcross progeny from Jj Bagg albino × Jj $F_1$ (Bagg albino × dba) matings.

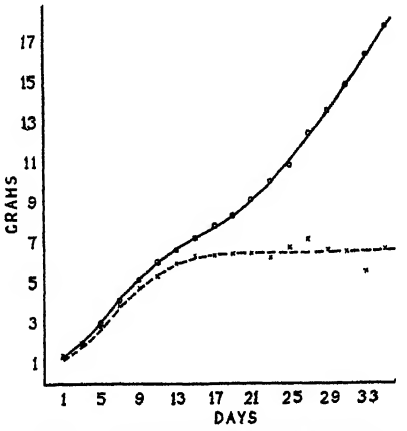


Fig. 3. Curves showing the growth of normal (solid line) and jittery (broken line) backcross progeny from Jj Bagg albino × Jj $F_1$ (Bagg albino × C57Blk) matings.

factors carried in the C57Blk strain. No other peculiarities were noticed among the backcross progeny of the Bagg albino × C57Blk crosses that would substantiate this possibility since the time of onset of the jittery symptoms, the appearance of the affected animals, and the mean age at death were not significantly different from those of the other two affected groups.

The growth curves of the three affected groups were very similar, rising closely parallel to those of the normal animals and then breaking sharply at about the

fifteenth day. At the time of death, the affected animals were lighter by more than a gram than at 15 days of age. This loss of weight was evident in the living animal and was closely correlated with the increasing intensity of the symptoms.

The exact day on which the jittery animals died was recorded in 29 cases. The mean in these cases was 31.89 ± 0.55, with a standard deviation of 3.009 days. No significant differences were found between the mean ages at death of the three groups of affected animals kept under daily observation.

The growth curves of the jittery animals showed a striking resemblance to those of dwarf mice. Snell (1929) pointed out that the latter could not be distinguished from their normal litter mates until fourteen days after birth, when they began to grow more slowly. The same condition was found in the dwarf rat by Lambert and Sciuchetti (1935). In the latter, there was a statistically significant difference between the weights of the dwarf and normal animals as early as five days after birth, but the deviation was not great until the twelfth day. The dwarf mutant in fowls cannot be separated from the rest of the brood until after fourteen days (Mayhew and Bailey, 1932). The case of hereditary tremors described by Huestis and Barto (1936) was not related to dwarfism, so far as is known, but the affected animals failed to grow properly after fourteen days and died before the end of the fifth week, as in the case of jittery. The reason for this coincidence is not known, but is of special interest since it has been shown that pituitary implants (Smith and MacDowell, 1930) or extracts from the anterior pituitary (Kemp, 1934) stimulate dwarf mice to gain weight.

## HEREDITY

The mutant, jittery, appeared in the sibship of ♂ 6 in the 37th generation of the Bagg albino strain. From the review of the ancestry of this sibship presented in table 1 it may be seen that, of the five members of this litter (♂ 6, ♀ 7, ♀ 8, ♀ 9, and ♀ 10), all were heterozygous except ♀ 7, showing that at least one of the parents of the 36th generation must have been heterozygous for jittery. None of the members of the litter, including ♂ 1, ♀ 2, ♀ 3, ♀ 4, and ♀ 5, produced affected offspring when mated to known heterozygous animals. The group of jittery animals described here were descendants of ♂ 6 and ♀ 8, ♀ 9, and ♀ 10.

Because the homozygous recessive animals died, it was impossible to produce a large stock by the conventional method of mating affected animals. To overcome this difficulty, the genetic constitution of each individual was tested by crossing it to a known heterozygote.

The test matings supplied a valuable index to the nature of the mutant involved, provided certain corrections were made. The data included only litters in which affected animals appeared, since only jittery litters revealed the genotype of the parents. When DR × DR matings were made, there were some litters in which no RR animals appeared. These should have been considered, but when data from progeny tests were used, the DR × DR matings that did not produce affected offspring could not be differentiated from the DR × DD matings and were, therefore, not included. The number of normal offspring from such matings should be too high by an amount depending upon the average size of the sibships involved. Since the mean litter size was known (table 4), a correction could be applied to overcome the difficulty. The following correction was given by Davenport and Ekas (1936):

$$W = \frac{p}{1 - (q)^s}$$

where $W$ = the percentage of RR animals expected, $s$ = the mean litter size, $p$ = the theoretical Mendelian ratio of RR, and $q = 1 - p$.

When the expected numbers of normal and jittery animals were calculated on the basis of the corrected percentages, the observed numbers came close to expectation. The percentage of affected animals was not significantly higher than expected, amounting to 29.81 per cent instead of 28.17 per cent as calculated. These data and the chi-squared values are given in table 5. The sex ratios were very close to expectation. These data indicate that the gene, jittery, is recessive and not sex-linked.

In matings of Jj Bagg albino females × Jj Bagg albino males, only animals proved

TABLE 4

LITTER SIZES AT BIRTH AND AT TWENTY-ONE DAYS AFTER BIRTH, AND THE MEAN NUMBER DEAD AT BIRTH AMONG THE PROGENY OF VARIOUS MATINGS

| Matings | No. of litters | Mean litter size | | Mean number per litter dead at birth |
|---|---|---|---|---|
| | | At birth | At 21 days | |
| Bagg albino JJ × JJ | 44 | 7 295±0 339 | 6 886±0 324 | 0 227 |
| Bagg albino Jj × Jj | 26 | 7 461±0 535 | 6 807±0 263 | 0 270 |
| Bagg albino, Test Jj × Jj | 74 | 7 594±0 204 | 7 216±0 127 | 0 324 |
| Backcross Jj × Jj (dba and C57Blk) | 18 | 9 222±0 430 | 9 167±0 410 | 0 000 |

TABLE 5

PROGENY OF Jj × Jj MATINGS

| Matings | | Normal | | | | Jittery | | | | Total | Chi-squared |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ♂ | ♀ | ♂+♀ | Per cent | ♂ | ♀ | ♂+♀ | Per cent | | |
| Bagg albino Test Jj × Jj | Obs. | 184 0 | 188 0 | 372.0 | 70.19 | 83 0 | 75 0 | 158 0 | 29.81 | 530 0 | 339 |
| | Cal. | 190 3 | 190 3 | 380.6 | 71.83 | 74 7 | 74 7 | 149 4 | 28 17 | 530 0 | |
| Bagg albino Known Jj × Jj | Obs. | 52 0 | 73 0 | 125.0 | 71.02 | 26 0 | 25 0 | 51 0 | 28 98 | 176 0 | 706 |
| | Cal. | 66 0 | 66 0 | 132.0 | 75 00 | 22 0 | 22 0 | 44 0 | 25 00 | 176 0 | |
| Backcross Jj × Jj (dba and C57Blk | Obs. | 62 0 | 71 0 | 133.0 | 73.08 | 23 0 | 26 0 | 49 0 | 26 92 | 182 0 | .165 |
| | Cal. | 68 2 | 68 2 | 136.4 | 75.00 | 22 8 | 22 8 | 45 6 | 25.00 | 182 0 | |

to be heterozygous for jittery were used, and all the litters were included, whether affected animals appeared or not. A few were excluded when, owing to some accident, a large part of the litter died within the first few days after birth. Of the 176 $F_1$ progeny from 26 litters, 51 were affected and 125 were normal. The percentage of affected animals, 28.98, was not significantly above expectation. The data are presented in table 5. The sex ratio among the affected offspring of the Bagg albino Jj × Jj crosses was very close to 1 : 1, as is shown in table 5, and, therefore, precluded the possibility of sex linkage.

Outcrosses were made for the purpose of testing the possibility that jittery was peculiar to the Bagg albino strain. Known heterozygous Bagg albino males were mated to unrelated females from inbred stocks of the C57Blk and dba strains. Eighteen of the $F_1$ females from these crosses were mated back to known heterozygous Bagg albino males. Eleven of the $F_1$ females produced litters containing

jittery animals, whereas the remaining seven produced only normal offspring. When only the Jj hybrids were considered, a 3 : 1 ratio was expected in the backcrosses. One hundred eighty-two backcross offspring were obtained from these matings, of which 49, or 26.92 per cent, were jj, approaching the expected ratio very closely (table 5). The same populations classified according to sex are presented in table 5, from which it is evident that there was no sex linkage.

The backcross data show that jittery was not peculiar to the Bagg albino strain. It is justifiable to speak of jittery as a recessive trait, since the affected animals appeared in these crosses in numbers closely approximating Mendelian expectation.

It is interesting to note the behavior of this gene with reference to the segregation of the various genes affecting coat color. The backcrosses were not designed to test linkage relations since albino animals were very unfavorable for this purpose and sufficiently large populations were difficult to obtain.

TABLE 6

COMPOSITION OF BACKCROSS PROGENY
BAGG ALBINO × $F_1$ (BAGG ALBINO × C57Blk)

| | Normal | | | | | Jittery | | | | | Total | Chi-squared |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CABD | CAbD | cc | Number | Per cent | CABD | CAbD | cc | Number | Per cent | | |
| Obs..... | 27 0 | 18 0 | 39 0 | 84 0 | 73 05 | 6 0 | 7 0 | 18 0 | 31 0 | 26 95 | 115 0 | 109 |
| Cal. . | 21 6 | 21 6 | 43 1 | 86 3 | 75 00 | 7 2 | 7 2 | 14 3 | 28 7 | 25 00 | 115 0 | |

Bagg albino = ccAAbbDDJJ.
$F_1$ (Bagg albino × C57Blk) = CcAaBbDDJJ.

The composition of the backcross progeny from the C57Blk females is shown in table 6. Only litters from known Jj $F_1$ females were considered. The number of affected animals produced in the C57Blk backcross amounted to 26.95 per cent of the total, which is very close to expectation.

Previous tests had shown that the genotype of Bagg albino animals hereterozygous for jittery could be written ccAAbbDDJj, whereas that of the C57Blk was aaBBCCDDJJ, and the dba strain was CCaabbddJJ. Both C57Blk and dba were homozygous for all the factors involved. The $F_1$ from the C57Blk outcross (Bagg albino × C57Blk) contained two genotypes, namely, CcAaBbDDJJ and CcAaBbDDJj. Backcross progeny from the JJ females were omitted since no jj offspring were produced, but the backcross progeny from the Jj females contained six classes (table 6). The numbers were small but were in reasonably close agreement with expectation for an autosomal recessive gene.

The $F_1$ population from the outcross of Bagg albino males heterozygous for jittery × dba females consisted of two genotypic classes but only one phenotypic class. They were CcAabbDdJj and CcAabbDdJJ. These could be differentiated only when the backcross generation appeared, since litters from the former contained affected animals whereas those of the latter did not. The litters in which affected animals appeared are presented in table 7. The observed numbers were close to the calculated ratios. The numbers of animals in two backcross generations were small for detecting linkage relations, but it was evident that the jittery gene was not closely linked with the c or b genes.

The possibility of accumulating modifying factors from the C57Blk and dba strains to which Jj Bagg albino animals were outcrossed could be checked by several

methods. The mean age at onset of jittery among the C57Blk and dba backcross groups did not differ significantly from that of the Bagg albinos. Similarly, no significant difference was found in the mean age at death between the Bagg albinos and the C57Blk outcross groups. These data (table 2) indicate that modifying factors affecting these two characters were not accumulated from the dba and C57Blk strains.

An inspection of the growth curves of the three groups of animals (text figures 1, 2, and 3) will reveal that the jittery C57Blk backcross progeny deviated from their normal sibs much earlier than the affected animals of the Bagg albino group or the dba backcross group. The deviation in the former group was significant after the ninth day, whereas in the latter two groups a significant deviation was not found until the seventeenth day. The normal progeny of the C57Blk backcross group which had the same genotype as their jittery litter mates, except for one j gene, did not

TABLE 7

COMPOSITION OF BACKCROSS PROGENY
BAGG ALBINO × $F_1$ (BAGG ALBINO × dba)

| | Normal | | | | Jittery | | | | Total | Chi-squared |
|---|---|---|---|---|---|---|---|---|---|---|
| | CAbD | cc | Number | Per cent | CAbD | cc | Number | Per cent | | |
| Obs. .. | 20 0 | 22 0 | 42 0 | 72 41 | 9 0 | 7 0 | 16 0 | 27 59 | 58 0 | 1002 |
| Cal. | 21 25 | 21 25 | 43 5 | 75 00 | 7 25 | 7 25 | 14 5 | 25 00 | 58 0 | |

Bagg albino = ccAAbbDDJJ.
$F_1$ (Bagg albino × dba) = CcAabbDdJJ.

differ significantly from the normal offspring of the other two groups. Therefore, the difference between the growth curves of the C57Blk backcross jittery animals and those of the jittery animals of the Bagg albino and dba backcross groups must have been due to modifying factors introduced from the C57Blk stock. The general character of the affected animals was not noticeably different in the three groups, but no method was available for measuring objectively the details of behavior.

Litter size might have been affected by modifying factors. The mean litter sizes at birth and at twenty-one days after birth among the combined backcross Jj × Jj matings were 9.22 ± 0.430 and 9.167 ± 0.410, respectively. Those of the Jj × Jj Bagg albino matings were 7.46 ± 0.535 and 6.807 ± 0.263. These differences are significant. It is, however, possible that the increased litter sizes from the backcrosses were due to heterosis rather than the action of the jittery gene. The evidence indicates that modifying factors affecting the rate of growth of the jittery animals were accumulated only from the C57Blk strain to which the Jj Bagg albino animals were outcrosses.

It might be expected that a lethal gene which caused the death of the homozygous animals within a short time after birth would also tend to reduce the size of the litters at birth, or before, as a result of a precocious lethal action. Evidence bearing on this point is presented in table 4. The mean litter sizes at birth and at twenty-one days after birth were not significantly different between the Bagg albino Jj × Jj and JJ × JJ matings. Similarly, the mean number dead at birth was not significantly higher in litters from Jj× Jj than in those from JJ × JJ matings. No precocious lethal effect of the jittery gene was demonstrated.

The possible genetic relationship between jittery and shaker-I was investigated

because these two mutant characters had some characteristics in common. Both arose in albino mice obtained from the colony of Dr. E. C. MacDowell. Tetany of the voluntary muscles is characteristic of jittery mice and was found in extreme cases of shaker-I animals by Lord and Gates (1929). In view of these similarities, experiments were undertaken to determine whether these two mutants were due to the same or allelomorphic genes.

A strain of shaker-I mice was obtained from the Roscoe B. Jackson Memorial Laboratory. Animals from this strain were crossed to known heterozygous jittery mice. If jittery and shaker-I were due to nonallelomorphic genes, the $F_1$ progeny would be expected to contain only normal animals, whereas if they were due to allelomorphic genes, affected animals should appear in one-half of the $F_1$ progeny. Eight matings were made between known heterozygous jittery and homozygous shaker-I animals. Sixty-nine $F_1$ progeny lived twenty-eight days or more, and all were normal, as would be expected if two nonallelomorphic genes were involved. The sh-Ij combination could not have been lethal since the mean litter size at birth from these crosses was 8.26. It was, therefore, unlikely that shaker-I and jittery were due to the same or allelomorphic genes.

Among the several hundred jittery animals observed in this study, only fifteen were classified as atypical. Three of these were clearly jittery, but lived beyond the age at death of typical affected animals. They were killed for experimental purposes on the 56th, 90th, and 97th days after birth. They were probably homozygous recessive (jj) animals that had escaped temporarily the lethal action of the jittery gene.

The remaining twelve atypical animals did not show the extreme muscular incoördination which characterized the typical cases. However, their sluggish behavior distinguished them from normal or typical jittery animals. Two of the females in this group reached breeding age and were mated to known Jj males. Their offspring included twenty-five normal and fourteen typical jittery animals. These twelve atypical cases may have been heterozygous (Jj) individuals which showed some effects of the jittery gene.

## PATHOLOGICAL STUDIES

*Parabiotic twins.*—If jittery was due to the presence or absence of a diffusible substance in the blood streams of affected animals, it should have been demonstrable by making parabiotic twins between normal and affected siblings. Six pairs of parabiotic twins were made according to the method of Burnster and Meyer (1933). The animals selected were always litter mates and, in all but the first pair, were of the same sex. The operation was performed as soon as possible after the symptoms of jittery appeared.

The range of the age at death for nonparabiotic jittery animals was 25 to 39 days. The affected animals in parabiotic union with normal sibs lived well beyond this range. The mean age at which parabiotic union was broken by the death of one of the members was 51.5 days. Details are presented in table 8.

None of the normal animals showed signs of jittery. The affected animals, however, were distinctly benefited since their lives were prolonged and tetany was eliminated. Each of them grew considerably and could not be distinguished in size from its normal twin. The affected animals were distinguishable by their peculiar crouching posture and incoördinated movements, but differences in size were slight.

The experiment indicated that no substance was passed from the affected to the normal animals in quantities sufficient to influence behavior, but that the reverse may have been true. This might have been the case if jittery had been due to a

deficiency of some sort. It was not necessarily true, however, since the affected animals did not live indefinitely; their lives were merely prolonged. It is possible that the mechanical support offered by their normal twins plus the increased opportunity to get food and water accounted for their improved condition. To test this possibility, feeding experiments with jittery animals were started.

*Feeding experiments.*—Three jittery animals which had shown the malady on the 14th day were selected. On the 15th day, they were placed with a lactating female whose own litter of eight had been removed so that the food previously consumed by eight mice was now available for three. No increase in weight was noticed, and the three animals died on the 28th, 29th, and 30th days, respectively, which were within the range of ages at death for affected individuals when in competition with their normal litter mates.

The muscular incoördination of the jittery animals may have prevented them from suckling properly even though there was no competition. This possibility was

TABLE 8

PARABIOTIC TWINS MADE BETWEEN NORMAL AND JITTERY ANIMALS

| Pair no. | Sex | Age (days after birth) at | | Animal first to die | Cause of death |
|---|---|---|---|---|---|
| | | Union | Death | | |
| 1 | ♂♀ | 20 | 57 | Normal | Accidental |
| 2 | ♂♂ | 19 | 55 | Jittery | ? |
| 3 | ♀♀ | 19 | 57 | Jittery | Accidental |
| 4 | ♀♀ | 20 | 46 | Jittery | ? |
| 5 | ♂♂ | 19 | 47 | Jittery | ? |
| 6 | ♂♂ | 13 | 47 | Jittery | ? |

checked by placing the affected animals in a small cage in which food and water were available at every point. Finely ground food was moistened and scattered over the floor of the cage every few hours, and overhead water bottles were arranged so that they could be reached from every point in the cage. Five 18-day-old jittery animals, which should have been able to feed independently, were placed in this cage. All lost weight and died within the range of ages at death of affected animals. Several other unsuccessful attempts were made to feed the jittery animals by hand. At autopsy, affected animals always showed some of the signs of inanition although their stomachs and intestines usually contained food.

These experiments indicate that the loss of weight was not due primarily to lack of food. They also suggest that the increased life span of jittery animals united to normal animals by parabiotic union was not the result of increased opportunity to get food and water, although it is possible that the affected animals received some additional food supplies through the blood stream from their normal twins. It is likely that the benefits resulting from the parabiotic union were due to substances obtained in small quantities from the normal twin, probably a hormone of some sort. Since pituitary and parathyroid deficiencies were known to produce some of the characteristics of the jittery animals, these hormones were tested.

*Parathryroid experiments.*—The similarity of the syndrome of parathryroid tetany to that of jittery was very striking, and in view of the results obtained from the parabiotic experiments an attempt was made to eliminate tetany by the administration of calcium salts. A series of twenty affected animals was used: five were

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given injections of sterile Ringer's solution, and the remaining fifteen were given a graded series of doses of 0.1 per cent calcium lactate daily for five consecutive days. No improvement was observed in any of the animals, and the tetany continued as before.

With the aid of Mr. H. C. Johnson, of the Department of Biochemistry, a series of tests was made to determine the actual calcium content of the blood of jittery and of normal mice at 20 days of age. Tests were run for diffusible calcium and for total calcium on separate groups of mice. The mean quantity of ionizable calcium as determined by the ultrafiltration method was 5.0 mg. per 100 cc. of serum for the six affected animals and 5.3 mg. per 100 cc. of serum for the seven normal ones. This difference is not significant. The total calcium was 10.3 mg. per 100 cc. of serum both in the eighteen normal animals and in the ten affected ones. These experiments indicate that the jittery animals were not abnormal because of a parathyroid deficiency and that the beneficial effects of parabiotic union could not be attributed to a parathyroid hormone.

*Pituitary experiments.*—It was pointed out by Smith and MacDowell (1930) that the dwarf condition in certain strains of mice could be partly overcome by implanting anterior lobes of the pituitary, and that the dwarf mice actually were deficient in the eosinophile cells of the anterior lobe. This result was confirmed by Kemp (1934), who was able to accomplish the same result by injecting preparations of the anterior lobe. The similarity of the growth curves of dwarf and of jittery mice has been pointed out, but the dwarf mice did not show the nervous symptoms of jittery. It seemed possible that the jittery mice were dwarf for one reason, such as a pituitary deficiency, and jittery for another.

To test this possibility, three jittery animals were implanted with two whole pituitaries each day. Implantations were made into the muscles of the hip. Each animal was weighed and observed daily to detect changes in weight or behavior. Implantations were started as soon as the affected animals could be identified and were continued until death. No appreciable differences in weight or behavior were noticed as between the implanted animals and the control or as between the implanted animals and the mean of the affected animals as a whole. All four died within the range for affected animals, namely, on the 30th, 31st, and 32d days. Since there was no indication that this line of attack would be profitable, it was not pursued further.

*Thymus experiments.*—It was noticed that the thymus glands of affected mice were much smaller than those of normal animals. Unlike man, the mouse does not normally lose its thymus by spontaneous involution. A number of adult mice from several strains were examined to check this point. The same observation was made by Masui and Tamura (1926). Rowntree, Clark, and Hanson (1934) had asserted that daily injections of a hydrochloric acid extract of calves' thymus into rats of successive generations caused the offspring to be more and more precocious in each successive generation. It was suggested that the thymus played some part in the development of the normal animal. The peculiar involution of the thymus in the affected animals and not in their normal litter mates suggested that perhaps it might be related to the cause of the malady.

To test this possibility, eleven affected mice were selected as soon as they could be differentiated from their normal litter mates and were implanted each day with one-half of the thymus from a normal mouse of about the same age. The implantations were made subcutaneously with a large Leur needle. Daily weighings and observations were made. Five of the eleven animals died before they were 30 days

old, three more before the 40th day, two on the 42d day, whereas one of them lived 94 days. The growth rate and general behavior did not differ significantly from those of the untreated jittery animals of the same strain, except that six of the eleven animals lived beyond the range of ages at death for untreated animals. The animal that lived for 94 days may have been an atypical jittery case similar to those described in a previous section. This cannot be proved, but the symptoms of jittery were still present in this animal as they were in all those implanted with thymus, in spite of the fact that life was prolonged. The thymus glands of the thymus-implanted animals involuted as though implants had not been given. Evidently such implants did not overcome the primary cause of jittery.

*Vitamin A studies.*—Nervous disorders have been reported in animals raised on diets deficient in vitamin A. Hart, Miller, and McCollum (1916) demonstrated lesions in the nervous systems of swine on low vitamin A diets. Mellanby (1926) showed that dogs kept on low vitamin A diets developed severe nervous symptoms with convulsions and paralysis of the hind legs. Myelin degeneration of the peripheral nerves was found in these cases. Hughes, Aubel, and Lindhardt (1928) reported muscular incoördination and spasms or convulsions in swine in cases of avitaminosis A. No complete paralysis was found, but the peripheral nerves showed myelin degeneration. Zimmerman (1933) observed incoördination and paresis in rats kept on a low vitamin A diet and was able to demonstrate myelin degeneration in the peripheral nerves and spinal cord. Sutton, Setterfield, and Krause (1934) studied this subject in rats and were able to produce the same symptoms by vitamin A depletion. Myelin degeneration of the peripheral nerves was also found.

The symptoms of avitaminosis A, except for the eye disorders, resembled those of jittery in many respects. The posture, emaciation, convulsions, decrease in weight, and eventual death were common to the two maladies. Five animals were selected as soon as they could be definitely diagnosed as jittery, and given 0.005 gram of carotene in butter each day until death. Sutton, Setterfield, and Krause (1934) found that 1 gamma of carotene daily was sufficient to relieve the nervous symptoms in the rat. No improvement was observed in the treated animals, and they died within the range for untreated jittery animals.

An atypical jittery animal which had attained the age of 59 days was available. This individual was fed the same dose of vitamin A, in addition to a regular diet of ground Fox Chow, for a period of 32 days, when it was accidentally killed. Daily weighings were made, but no improvement was observed.

*Fostering experiments.*—Greene, Hu, and Brown (1934) stated that "rapid improvement follows fostering" of their dwarf rabbits on unrelated normal foster mothers. The possibility of a similar action in the case of jittery was tested by fostering entire litters from three heterozygous matings on unrelated lactating Strain A females. The litters were removed from their own mothers at birth and given to their foster mothers. Daily observations were made and each animal was weighed each second day, beginning at birth. The three litters contained eight affected animals.

No improvement was observed in the jittery animals of the fostered group. When their daily weights were compared with those of unfostered jittery animals, it was found that they had lost weight more rapidly than the unfostered jittery ones. Death occurred in each case within the range for unfostered jittery mice of the same strain.

*Starvation experiments.*—At autopsy, the jittery animals showed the general symptoms of inanition. The flaccid condition of the intestine, the pale color of the

liver and spleen, and the extreme emaciation of the animals seemed to indicate that starvation in some way played a part. It has been shown that increasing the food intake of the affected animals did not prolong their lives or improve their condition. However, since a few of the heterozygous individuals had shown some of the symptoms of jittery spontaneously, it is possible that heterozygous animals could be induced to show the trait by starvation.

Seventeen offspring about 15 days old were taken from heterozygous matings and fed on a diet of Fox Chow and water that was just sufficient to maintain life. On the basis of chance, two-thirds of these nonjittery animals would have been heterozygous. While it was impossible to distinguish the heterozygous from the homozygous normal animals by inspection, it was very unlikely that all of them belonged to the latter class. On the 15th day, the mean weight of the group was 8.04 gm. Seven days later, when three of the animals had died from starvation, the mean weight was 6.02 gm. None of the animals showed any of the signs of jittery except loss of weight. At autopsy, however, the same signs of inanition were present in the starved animals as normally appeared in the jittery ones.

## GROSS PATHOLOGY

At autopsy, the jittery animals showed typical signs of extreme inanition and emaciation. Their bodies were thin, and the tail vertebrae and the bones of their feet were clearly outlined since the skin was drawn tightly about the skeletal structure. The proportions of the body seemed normal, as though the growth processes had stopped generally. The skin appeared dry but not wrinkled, and in the albinos it was of a darker color than is normal for these animals. The intestines always contained food but were noticeably lacking in muscular tone and were very dark in color. No lesions were found in the intestines or stomachs. The livers appeared to be of normal size, in proportion to the rest of the body, but were light in color. On section, they presented dry, smooth surfaces, free from lesions, and showed the lobules clearly. The spleens were occasionally smaller than normal and lighter in color. The kidneys appeared normal when cut and no signs of congestions were seen. Very little fat was found. The hearts were not enlarged, but appeared flaccid. No congestion was seen in the lungs, and they would float in water. In the later stages of the disease the thymus glands were very small or lacking. The nervous systems appeared normal although the brains and cords were smaller than usual. The meninges were free and not unduly thickened. The pituitary bodies were congested but did not appear larger than normal; however, no actual measurements were made. The thyroid glands showed no external peculiarities. The adrenal glands were normal in size, shape, and color. An examination of the reproductive structures revealed no abnormalities except in color and general muscular tone.

## MICROSCOPIC PATHOLOGY

Of the tissues studied microscopically, only those from the thyroid, pituitary, thymus, and nervous system showed changes which might have been associated with jittery. These will be discussed in detail.

*The thyroid gland.*—Plate 2, figure 5, shows a photomicrograph of the thyroid from a jittery mouse 11 days of age. It is normal in every respect. The cells of the follicles are cuboidal to slightly elongated and their nuclei are round and medially placed. Follicles are plentiful and filled with colloid, which shows no sign of excessive storage. Small drops of freshly secreted colloid are found at the lumen end of the cells. The rest of the colloid stains uniformly.

The thyroid gland shown in plate 2, figure 6, was taken from a jittery animal, 33 days of age. The follicles are present in normal numbers and almost normal size, but the colloid is less homogeneous and shows signs of retention. A few small drops of freshly secreted colloid are present. The secreting cells are low, and some are flattened to the proportions of a squamous epithelium. The nuclei are compressed and bulge the cytoplasm into the lumen of the follicle. Connective tissue hyperplasia and white cell infiltration are lacking. The thyroids from advanced cases were suggestive of hypoactivity.

Since these changes in the thyroid glands could not be detected until from seven to ten days after the appearance of jittery symptoms, and since the thyroid glands of starved normal animals were hypoactive, there was no proof that the hypoactive thyroid glands of the jittery animals were primarily concerned with the malady.

TABLE 9

MEAN CELL COUNT PER UNIT AREA (ONE UNIT AREA = 49,000 $\mu^2$) FROM THE ANTERIOR LOBES OF PITUITARIES OF JITTERY AND NORMAL MICE

| Normal | | | Jittery | | |
|---|---|---|---|---|---|
| Sex | Age | Mean cell count | Sex | Age | Mean cell count |
| ♂ | 11 | 212 3 | ♂ | 11 | 243 3 |
| ♀ | 20 | 242 4 | ♀ | 11 | 283 2 |
| ♂ | 30 | 162 6 | ♀ | 10 | 298 6 |
| ♂ | 45 | 205 6 | ♀ | 24 | 294 4 |
| ♀ | 38 | 174 0 | ♀ | 26 | 304 8 |
| ♂ | 24 | 233 6 | ♂ | 39 | 267 5 |
| | | | ♀ | 38 | 245 5 |
| Mean | 29 5 | 205 08 | Mean | 27 7 | 276 7 |

*The thymus.*—The thymus gland of the mouse does not normally undergo involution. Masui and Tamura (1926) state that it attains full development at two months of age and remains this size for the life of the animal. Our autopsy findings confirm this. The thymus glands of jittery animals began to undergo involution shortly after the appearance of the jittery symptoms. Within ten days involution was well advanced.

Plate 1, figure 3, presents a photomicrograph of the thymus of a normal 16-day-old mouse. The cortex and medulla of the gland are normally arranged and are composed of the typical constituents. In contrast to this, the thymus from a 33-day-old jittery animal is shown in plate 1, figure 4. The medulla is filled with cell debris and pyknotic nuclei, making it appear dark in the picture. The cortex is represented by a mass of stroma cells from which most of the lymphocytes normally found in this region had disappeared.

The involution of the thymus gland was not coincident with the appearance of the symptoms of jittery. There is no proof that these changes were other than secondary, since starved animals showed a partial involution of the gland and since implants of it did not overcome the disease.

*The pituitary.*—Serial sections, measuring 4 $\mu$, of the pituitary bodies of normal and affected mice were prepared and stained with Harris's hemotoxylin and eosin.

The lack of a satisfactory tinctorial method of differentiating chromophobe from basophile cells in the mouse pituitary precluded differential counts.

The anterior pituitary lobes of the jittery mice were congested and considerably more cellular than those from normal mice of the same age and sex (pl. 2, figs. 7 and 8). Cell counts were made with a ruled ⅞-inch ocular counting disk which marked out an area of 49,000 square micra on the slide when the oil immersion lens was used.

The mean number of cells for six normal and seven jittery mice is presented in table 9. The mean number for all affected animals was 276.70, and that of the normal mice, 205.08. The two groups did not overlap since the range for normals was 162.6 to 242.4, and that of the affected animals, 243.3 to 304.8.

The increase in the cellularity of the anterior lobes of the pituitaries of affected animals was probably greater than the figures indicate, since the blood passages were greatly distended with blood and, therefore, occupied a greater portion of the volume of the organ than in normal animals. The pituitaries of affected animals did not appear larger than those from normal animals, nor were mitotic figures abundant. On the contrary, the individual cells of the pituitaries from affected animals possessed only scant cytoplasm and their nuclei were hyperchromatic. The number of eosinophile cells did not seem to be decreased in the affected animals, but actual counts were not made.

The changes in the pituitary did not appear concurrently with the jittery symptoms. They were detected only after several days had passed. It was, therefore, impossible to attribute the physiological cause of jittery to the pituitary since affected animals implanted with whole normal pituitaries had failed to show improvement.

*The nervous system.*—The nervous system was studied by a number of methods. Serial sections were made of the brain and several regions of the cord in an effort to locate lesions, but none were found. The Marchi reaction as modified by Swank and Davenport (1935) was tried on the whole central nervous system. No positive results were obtained that would indicate myelin degeneration.

With slides of normal and degenerated nerves as standards, the peripheral nerves and spinal cord of affected mice were examined by the polarized light method of Setterfield and Baird (1936), but no signs of degeneration were detected.

Nissl's method was used on the cells of the cord in an attempt to detect in the neurones degenerative processes which might have been correlated with the jittery condition. In the later stages of the disease, the motor cells of the anterior horn in the lumbar region showed a vacuolated condition which, according to Penfield (1932), is indicative of degeneration. In the early stages of the disease, the vacuoles were not always found. The Nissl substance was peripheral in many of the cells and the nuclei were pushed to one side, showing central chromolysis. The degree of vacuolization was variable, being very extensive in some cases and hardly noticeable in others. One of the usual signs of degeneration was missing, however, namely, the migration of the glia cells into the region of the damaged neurones. This would indicate that the damage was recent and not of long duration. Plate 2, figures 9 and 10, shows photomicrographs of anterior horn cells from normal and affected animals.

Since it was not possible to demonstrate degenerative processes before the onset of the external symptoms, one could not be certain whether the lesions represented the primary cause of the disease or were merely secondary. Starved normal animals did not show degenerative changes in their motor cells. These changes in the jittery animals could not, therefore, be attributed to inanition.

## DISCUSSION

No traits have been mentioned in the literature which are strictly comparable to jittery in mice. Perhaps the most similar one is congenital palsy in guinea pigs reported by Cole and Ibsen (1920). Here the tetany was present at birth. Since no pathological findings were given, the comparison cannot be carried beyond this point. A similar situation was found in the case of hereditary tremors in *Peromyscus* described by Huestis and Barto (1936). In this case, the tremors did not appear until the animals were 14 days of age, and tetany was not mentioned. Both of these cases, like jittery, were due to recessive lethal genes.

Degeneration of the motor cells is characteristic of both jittery in mice and paralysis in dogs, but in the latter case the paralysis is limited to the posterior limbs, whereas in the former tetany is general. Jittery is due to a single recessive gene, whereas paralysis in dogs is probably due to at least three dominant genes.

A slight but not significant excess of affected animals was found in each group of heterozygous matings. This excess was probably due to the appearance of a few cases in which genetically heterozygous animals showed some of the jittery symptoms and were classified as homozygous recessive individuals. Two such cases were proved to be heterozygous by breeding tests, and ten others were suspected.

The attempt to determine the physiological mechanism by which the jittery condition is produced has met with little success. Since the affected members of parabiotic twins increased in weight and showed no tetany, it would seem that they received beneficial substances from their normal twins; but none has been identified. The failure of pituitary implants to benefit affected animals indicates that the beneficial effect of parabiosis is not due to a pituitary hormone. The lack of pathological changes in the reproductive organs would tend to confirm this. The increased cellularity and congestion of the anterior lobes of the pituitaries of jittery mice may have been secondary effects.

The thyroid glands of jittery mice were hypoactive. Starved normal mice also developed hypoactive thyroids. The changes in the jittery thyroids could not be attributed definitely to a pituitary deficiency. No evidence was found that the thyroid was primarily concerned with jittery. The affected mice did not resemble cretins and the thyroid changes did not appear until late in the course of the disease.

It has been shown experimentally that jittery is not due to a parathyroid deficiency, at least so far as its relation to calcium metabolism is concerned. Moreover, there is no histological evidence of their abnormal behavior.

The beneficial effects of parabiosis were not due to a substance produced by the thymus, since the nervous symptoms were not relieved when whole thymus glands were implanted into affected animals. The involution of the thymus in jittery animals may have resulted from inadequate food intake, since a partial involution of that organ was produced by starving normal animals; on the other hand, it could have been produced by a hormone imbalance. Most workers agree that the removal of the pituitary leads to an early involution of the thymus (Ascoli and Legnani, 1912; Houssay and Lascano-Gonzalez, 1934; Houssay and Hug, 1921; Kapran, 1932; Smith, 1930). Kemp (1934) stated that the administration of anterior pituitary extracts to dwarf mice caused a proliferation of the stroma of the cortex of the thymus, whereas Marine, Manley, and Baumann (1924) stated that involution of the thymus also followed removal of the thyroid. It is evident, therefore, that the hormones of the glands which showed the pathological changes in jittery mice are capable of bringing about all of the changes seen in the thymus glands.

The degenerative changes in the nervous system may have been primary, but they did not appear early in the history of the disease. They might equally have been the result of a primary cause elsewhere in the body of the animal, since it is well known (Penfield, 1932) that nerve cells are easily affected by organic changes outside of the nervous system. It is uncertain, therefore, whether jittery is due to a degenerative process in the nerve cells, to endocrine disturbances, or to some other cause not discovered.

## SUMMARY

1. A new mutation in the house mouse, called jittery, is described. The affected animals were normal until about the 12th day, when symptoms of muscular incoördination appeared, followed by tetany, loss of weight, extreme emaciation, and finally death at a mean age of 31 days.

2. Genetically, the defect is due to an autosomal recessive lethal gene.

3. Jittery animals united by parabiotic union to normal litter mates showed no tetany, attained a larger size, and lived much longer than the jittery controls. Similar results were not obtained, however, by forced feeding, pituitary implants, thymus implants, feeding carotene, or injecting calcium salts. The calcium content of the blood of affected animals was normal.

4. Microscopically, the pituitary showed an increased number of cells with a correspondingly smaller mass of cytoplasm, hyperchromatic nuclei, and engorged blood passages. Eosinophile cells were present, but the proportion of each cell type present was not determined.

The thyroid gland became hypoactive in the later stages of the disease, and the thymus, which is normally persistent in mice, underwent involution.

Motor cells in the lumbar region of the spinal cord showed varying degrees of vacuolar degeneration, but these changes were not coexistent with the first appearance of the external symptoms.

Starvation produced a hypoactive condition of the thyroid gland and a partial involution of the thymus, but not the characteristic changes of the pituitary nor degeneration of the motor cells.

5. The immediate cause of jittery cannot be attributed, with certainty, to any of these pathological conditions.

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# PLATES

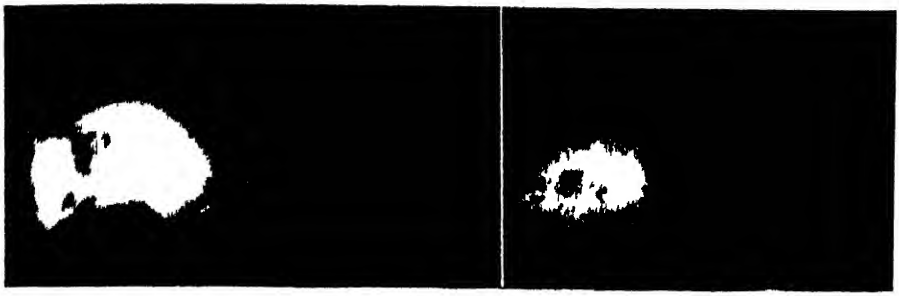
## PLATE 1

Fig. 1. Photograph of a normal mouse at 29 days of age.

Fig. 2. Photograph of a jittery litter mate of the mouse shown in figure 1.

Fig. 3. Photomicrograph of the thymus of a normal mouse at 16 days of age, showing the normal arrangement of parts. × 43.

Fig. 4. Photomicrograph of the thymus of a jittery mouse at 33 days of age, showing involution. × 43.



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## PLATE 2

Fig. 5. Photomicrograph of the thyroid gland of a jittery mouse 11 days of age. The gland is normal in structure. × 500.

Fig. 6. Photomicrograph of the thyroid gland of a jittery mouse 33 days old, showing the deeply staining colloid and flattened epithelium. × 500.

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Fig. 10. Photomicrograph of a number of motor cells from the lumbar region of a jittery mouse 29 days of age. The cells show vacuolar degeneration and chromolysis. Nissl's stain. × 500.

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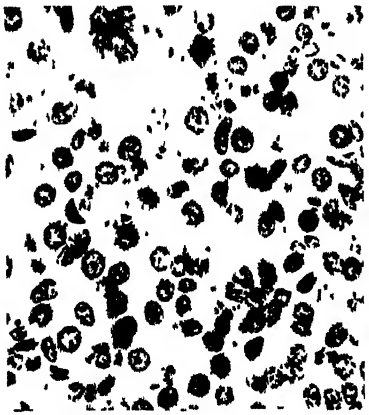

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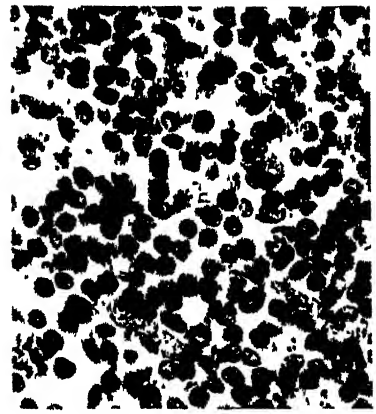

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# THE FLAGELLATE SUBFAMILY OXYMONADINAE

BY

JOY BARNES CROSS

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# THE FLAGELLATE SUBFAMILY OXYMONADINAE

BY

JOY BARNES CROSS

## INTRODUCTION

THE SUBFAMILY Oxymonadinae consists of certain xylophagous flagellates living as mutualistic symbionts in the gut of perhaps all of the termites of the family Kalotermitidae. The geographical distribution is world-wide in tropical and subtropical regions. A range of such extent suggests a high degree of diversification among the flagellates and the supposition is amply justified. Many of these flagellates have single nuclei, but some have more than a hundred. In the multinucleate forms, the nuclei may be either distributed haphazardly or limited to a definite pattern. The extranuclear organelles also occur singly or in large numbers and may differ in arrangement and proportion. Cyclical changes which present added complications include alterations in the appearance and position of the nucleus, degeneration and replacement of extranuclear organelles, and simulation of the immature form of one genus by the mature form of another. Adaptations to an alternately motile and sessile stage result in definite dimorphism in which the body varies from spheroidal to irregular elongate forms with long tubular attachment organelles; and in which the maximum length is frequently five or six times the minimum.

Although diversification among species and variation within species are expressed in a multiplicity of forms, all of the Oxymonadinae are evolved from an essentially simple unit consisting of a nucleus, an axostyle, a rostellum, and two blepharoplasts from each of which two flagella originate. Diversification has been achieved by repetition of the unit; by variation in length, breadth, and stainability of the different parts of the axostyle; by changes in the position of the nucleus in relation to the axostyle; and by changes in the relative sizes of the karyosome, the halo which surrounds the karyosome, and the nucleus. The blepharoplast-flagella complex exists practically unchanged throughout the subfamily.

Both the morphological unit and the methods of achieving diversity are simple, but the results are interestingly complex (text fig. A) and establish the flagellates of the subfamily Oxymonadinae as valuable subjects for evolutionary and cytological investigation. Because of the firmness with which they are attached to the chitinous lining of the termite gut, a knowledge of their functional pattern is of value for the problems of ecdysis and of the faunation of new colonies in the termite. Farther afield, the precision with which the complex fibrous organelles are periodically originated and destroyed affords an opportunity for morphological studies of the formation of intracellular fibers; and the shifting from a uninucleate to a multinucleate form in the evolutionary development of the Oxymonadinae permits a comparison with a pattern of cellular behavior long recognized as pathologic.

I am grateful to Professor Harold Kirby for specimens from his collection for the studies presented here, and especially for his advice and criticism of the manuscript and the illustrations.

## MATERIALS AND METHODS

Since all the slides are from Professor Kirby's collection, his method of recording them (1941*b*) has been followed. "... certain slides bearing each new species have been designated as 'syntype' slides, all derived from the same colony of termites... Preparations selected from the same host species of termite but not necessarily the same colony as the syntype, are designated 'xenosyntype' slides. Slides from other termite species bearing the same flagellate species... are denominated 'homosyntype' slides." TP- indicates that the accompanying number refers to a slide; and T- preceding a number refers to the file of records of termite colonies.

The most intensive studies have been made of specimens in smears, the usual sort of preparation used in the study of termite protozoa. Ordinarily, one termite intestine furnishes thousands of specimens for one smear. The term "thousands" is used advisedly, for the average number of protozoa occurring in a single section, 12$\mu$ thick, from a serial from the gut, as computed from ten sections of *Kalotermes minor* (Cross, 1941) exceeds fifteen hundred. However, oxymonads do not ordinarily appear in large numbers in smears and a comparison of the number in a section from the gut and in a smear shows that many of them must have been destroyed with the gut when the smear was made.

In addition to the usual recordings of the position of the specimen in the smear, rough sketches were made of all the recorded animals to facilitate subsequent examinations and appropriate classification into groups for later study. In general, each figure in the illustrations is representative of a considerable number of similar, registered specimens, but a few solitary specimens of peculiarly interesting or extraordinary forms have also been pictured. An 8 × or 15 × ocular with a 100 × objective has been used for the observations, and in a few instances a 20 × ocular was used for camera lucida drawings, giving a maximum magnification of 3400 ×.

Because of the extreme variation in size, the computation of measurements of central tendency in the Oxymonadinae was puzzling. Connell (1930) and I (1941) obtained separate averages for the large attached forms and for the small motile animals. The median has been used for the four oxymonads studied intensively in this paper because in the presence of extreme variability, it is a more reliable measure of central tendency than the arithmetical average. For the sake of ease in calculation, fifty-one animals were measured. When the measurements were assembled preparatory to the determination of the median, it was evident that the nuclear measurements were much less variable than those of the body. Since the ratios of the smallest to the largest body lengths in *Oxymonas grandis*, *O. megakaryosoma*, and *O. notabilis* were 1/6, 1/5, 1/8, and for the respective nuclear lengths 3/8, 1/2, 2/5, a ratio based on nuclear measurements would be the more dependable. The median of the

breadth of the karyosome compared with the median of the width of the nucleus (indicated throughout this paper as K/N) furnished a ratio which proved to be a remarkably close measure. The ratio of the width of the halo to the breadth of the karyosome (H/K) also proved reasonably constant. All of the ratios were computed from specimens, excepting those for *Oxymonas projector, O. pediculosa,* and *O. gracilis,* in which they were estimated from the figures given by Kofoid and Swezy (1926*a*). The width of the halo was determined by measuring the distance between the inner and outer circumferences of the clear ring which surrounds the karyosome and is not to be confused with the "diameter" of the halo which is a measurement that Zeliff (1930*a*) used. All measurements of the length of the body excluded the rostellum. The axostyle shoulder was used as the anterior locus except in *O. notabilis,* in which the position had to be estimated because the shoulder is retracted.

Only a small number of living specimens has been used. However, these included one colony of *Kalotermes minor* which was maintained for more than a year in a refrigerator dish on which the glass cover was prevented from closing tightly by inserting a small paper wedge. From time to time food was supplied by adding coarse towel paper, folded into thick pads and moistened with distilled water (May, 1941). This was an easy and safe method of feeding and of controlling moisture within the chamber. Observations on this colony and on a few specimens of *O. jouteli* from *Kalotermes jouteli,* Key Largo, Florida (T-4616), were made with darkfield illumination on smears in 0.65 per cent NaCl just as has been described earlier (Cross, 1941); but no new facts were learned.

Gut sections of *Neotermes howa* were available in Professor Kirby's collection; but intestines of *N. dalbergiae, N. tectonae, N. howa* var. *mauritiana,* and *Glyptotermes* sp. nov. from Uganda that had been fixed in Hollande's, Flemming's, or Schaudinn's solutions and stored in 80 per cent alcohol for several years were prepared for this study. Tertiary butyl alcohol was used for dehydrating and in combination with the paraffin for preliminary infiltration because it diminished the difficulties in sectioning the chitinous intima and the wood particles. Caution was exercised to insure the complete evaporation of the alcohol before embedding. One-tenth of its weight of white beeswax was added to the paraffin. The entire intestine was left intact up to the time of embedding, when the anterior portion was discarded after being dissected out with heated needles. This obviated the cutting and mounting of serials from regions of the intestine in which protozoans are not found. Amputation in the warm paraffin prevented leakage of the specimens, which would have resulted from earlier dissection in fluid reagents. Because the termites had not been fed previously on filter paper to decrease their wood content, the consequent grit in the paraffin blocks made the cutting of serial sections thinner than 12$\mu$ inadvisable.

The gut sections were stained with Delafield's (with or without a counterstain of eosin) or Heidenhain's haematoxylin after fixation with Hollande's or Schaudinn's solutions. After Flemming's fluid, Regaud's haematoxylin

was usually used. In a few slides, acid fuchsin was used as a counterstain following Heidenhain's haematoxylin. Delafield's haematoxylin, followed by eosin, was a good choice for gut sections in the study of *Oxymonas minor* and *O. grandis,* but was poor for smears because it does not stain the axostyle (Kirby, 1928) nor does it define the spindle structure as satisfactorily as the iron stain does. In gut sections for *O. megakaryosoma* and *Barroella coronaria,* Heidenhain's haematoxylin following Schaudinn's fixative was the better combination, and was also the most generally useful with all of the smears.

About four hundred serial slides were made from *Neotermes dalbergiae* and *N. tectonae,* because the large size of *Oxymonas grandis* promised an advantage in studying the mechanism of the holdfast. Termite colonies were chosen for the serials from which smears had shown either division stages or the dark-colored spherules called "chromidia" by Zeliff (1930*a*). One or two slides from each serial of an intestine were laid aside. The others were stained and searched swiftly for indications of "chromidia." This method provided about twenty unstained, "chromidia suspected" sister slides for a Feulgen reaction.

The de Tomasi (1936) adaptation of the Feulgen method was used. Following a notation in Reichenow (1927–29) that occasionally the aldehyde derivatives of thymo-nucleic acid were already present, hydrolysis was omitted with three slides. The staining reaction ("strong" Feulgen for 1½ hours) was negative. Two slides were hydrolyzed at 63° for 4 minutes. The reaction was negative for *Oxymonas.* Gut sections hydrolyzed at 63° for 5, 6, 9, and 10 minutes, respectively, gave positive reactions with the stain. The best results occurred at 9 minutes. The sections which had given negative results for *Oxymonas* without hydrolysis were hydrolyzed for 8 minutes and those which had been hydrolyzed for 4 minutes were hydrolyzed for an additional 4 minutes, after which the staining reaction was positive in both cases. Five per cent methyl green in 95 per cent alcohol was used as a counterstain.

There was no evidence of extranuclear chromatin, the so-called chromidia. With the longer periods of hydrolysis, both the wood particles (Lee, 1937) and the chitinous lining of the intestine gave positive reactions. (This similarity in chemical behavior suggests that the degenerating chitinous intima may have a nutritive value for the termite and the protozoa during the nonfeeding period of ecdysis.)

The pale, mustard yellow spherules found in the Oxymonadinae darken readily with iron-haematoxylin, do not stain readily with fuchsin, and resist staining with eosin. These reactions are not specific but suggest the possible presence of volutin. Following McClung (1937), sections of gut from *Oxymonas grandis* were stained deeply with carbol fuchsin and destained with aqueous iodine potassium iodide. If volutin is present, it retains the stain and the other cellular components are bleached. Since the spherules lost the color completely, long before any other structures were affected, the result of the test must be considered negative. Consequently, the application of the term "volutin" to the spherules found in *Oxymonas* is not warranted on the present evidence.

## SPECIAL STUDIES OF MORPHOLOGY

### OXYMONAS GRANDIS CLEVELAND[1]

*Oxymonas grandis* occurs in the intestine of *Neotermes dalbergiae* and *Neotermes tectonae* from Java and Sumatra. This report is largely an amplification of Cleveland's (1935) account, which was limited somewhat to his essential interest in the achromatic intranuclear figure, as his title implied. He described *O. grandis* as an excellent choice for study because the body and nucleus are larger than has been reported for any other oxymonad, and the value of that advantage has been extended by the discovery in *O. grandis* of counterparts for characteristics that were too small to interpret definitely in the small species. In addition to giving a more detailed account of the extranuclear organelles and of their behavior during mitosis, I have made some modifications in Cleveland's report of the mitotic nucleus. Certain differences in my observations are probably the result of my avoidance of specimens for mitotic studies that had been stained with Delafield's haematoxylin, because it either does not stain the cablelike portion of the intranuclear spindle or stains it faintly. Cleveland reported his use of that stain and a number of his illustrations show only the characteristics that it depicts.

Many of the structural variations found in *O. grandis* are most readily interpreted as periodical adaptations to an alternately motile and sessile period with the subsequent adjustment necessitated by a host-parasite relationship. A detailed account of a life cycle that approximates that of *O. grandis* has been given for *O. minor* in an earlier paper (Cross, 1941), and agrees in the more important aspects with that outlined by Connell (1930) for *O. dimorpha*. Briefly, it consists of a flagellated motile period, in which the body of the animal is relatively small and broadly ovoidal or spheroidal, and the anterior portion of the body is extended only slightly in a low mound to form the rostellum. The latter organelle, with its apical holdfast, serves for attachment, during the sessile period, to the chitinous intima lining the termite gut, and may be either short or several times as long as the body; either tubular or somewhat flattened, narrow or comparatively broad. The greatest variations in the size and shape of the body occur in the attached period.

Presumably, the small, motile form of *Oxymonas* is present following ecdysis in its host. Attachment is made to the intestinal intima, and growth and reproduction take place until another ecdysis disrupts the anchorage of the flagellate. Usually, the protozoan contents of the termite gut are lost with each moulting and refaunation takes place by proctodeal feeding, but at the close of the seventh instar the flagellates are retained through ecdysis and supply the alates with a fauna for the new colonies (Child, MS; Cross, 1941; May, 1941).

In sections of the intestine of *Kalotermes minor*, *Oxymonas minor* has been seen to form a relatively uniform peripheral banding of the lumen, but in sections from other Kalotermitidae the distribution has been less regular, and the somewhat discontinuous banding shown for *O. grandis* in a cross section

[1] See below, p. 112, for diagnosis and taxonomic position.

of gut from *K. dalbergiae* (pl. 8, fig. 36) is more typical. In it, *O. grandis* shares the outermost concentric circle with *Caduceia kalshoveni,* and the central region is composed of *Foaina nana, Devescovina parasoma,* and a few *Calonympha.* Frequently, in regions where the lumen is smaller, *O. grandis* has been the sole occupant, forming a continuous band, and occasionally the situation has been duplicated in *Glyptotermes* sp. nov. from Uganda with *O. megakaryosoma.* Oxymonads have been the only protozoans in the termite that I have seen appearing constantly in this peripheral ring, except in *Kalotermes flavicollis* where the distribution of both *Hexamastix* and *Tricercomitus* resembles it.

Gut sections show that the bodily deformities appearing in smears cannot always be considered artifacts. Since the cytoplasmic irregularities often conform to the interstices formed by the crowding bodies of adjacent protozoans, it seems probable that distortion is caused by crowding and bodily plasticity. Faulty fixation cannot be suspected because the large calonymphids retain their normal forms. Janicki (1915) reported that the "major" form of *Stephanonympha silvestrii* was more subject to distortion than the small "minor" form and this dissimilarity in behavior is equally true for the large *O. grandis* and the small *O. minor.*

Apparently, the presence or absence of an extended rostellum also depends in part upon the extent to which its neighbors crowd the animal from its point of attachment. A lengthy rostellum probably results from a passive growth adaptation to external pressure acting on plastic cytoplasm, and a complex neuromotor system is not needed to explain its morphology. Fowell (1936) stated that the neuromotor system of ciliates and flagellates is "almost certainly skeletal (at least) in part." The rostellar fibers are passively long or short as the growth in length of the cytoplasmic protuberance forming the rostellum requires. The active extension or retraction of a rostellum has been reported by no one except Nurse (1945), who stated that "the flagellates remained stationary but were seen to retract the proboscis." Since *Oxymonas* rarely "remain stationary" except during the period preceding disintegration, it seems probable that this so-called retraction was actually a degenerative change. At least, Connell (1930) observed that an extended organelle remained unchanged in spite of strong stimulation by a needle. The lengths of the rostella were not measured because their extreme and fortuitous variability deprives the measurements of significance for either cyclical phenomena or speciation problems.

The fibers of the rostellum, instead of contracting into a "sleeve" as Kofoid and Swezy (1926) assumed, are frequently coiled (pl. 6, fig. 19). In all of the specimens that I have seen, whenever the body of the oxymonad was coated with bacteria, the rostellum was equally coated. It is difficult to imagine what would become of these external parasites if the rostellum were suddenly drawn inside the body or why "hirsute" animals in which the recently extruded rostellum is still "bald," or at least only sparsely covered, have not been found if the rostellum can be thrust out suddenly and lengthily.

Cleveland (1935) described the rostellum as "... an extension of the body

made by the non-staining portion of the axostyle." This is possibly true in the early reorganization period, when the rostellum is a low mound, but his definition would more nearly conform to my observations if the word "occupied" were substituted for the word "made." In the serial sections from intestines of the termite, there is abundant evidence of crowding and crushing that could encourage the adaptive growth of such an organelle, and there is no visible evidence of the rostellar fibers actively pushing out a lengthy rostellum. Possibly Cleveland intended a less active type of extension by growth, but even that does not explain instances where the old holdfast and the old rostellar cytoplasmic protuberance have remained during cell division, and where the new rostellar fibers burrow forward to reoccupy a region that had existed previous to the origin of the fibrils (pl. 6, fig. 20). Consequently, Cleveland's figures B-6 and B-7, cannot be accepted as showing that ". . . extension of the body by the fibers is just beginning," but are to be interpreted as animals in which the holdfast is not yet developed and in which the rostellum is short and may or may not remain so.

Studies of live material demonstrate the unlikelihood of a passive crowding such as occurs in growing cells in metazoan tissue. Instead, the fluid in which the termite protozoa are suspended seethes with violently lashing bodies. Some of the protozoans in the termite gut are propelled by a varying number of flagella which range from a few to hundreds. In others, locomotion is achieved by forceful swimming strokes made by the rhythmical repetition of a swift and sharp bending of the body, followed by a slightly slower straightening. Because the axostyle in *Oxymonas* plays so active a part in this process, it is a temptation to overlook the contractile qualities inherent in the cytoplasm and to describe the axostyle as the causal agent of bodily motion. Locomotion in *Oxymonas* probably results from the combined activity of the flagella, the axostyle, and the body; and even in the sessile period the axostyle and the body continue their concomitant movements.

Perhaps within the crowded limits of the gut the activity of the protozoans is lessened, but their capacity for violent movement and consequent sudden changes in pressure cannot be questioned. Marsland (1939*a*, *b*) and Pease (1941) found that appropriate increases in pressure can produce a homogeneous sol within a cell so that dissolution of the mitotic figure takes place, streaming in *Elodea*, and furrowing in dividing egg cells can be stopped temporarily. Although the amounts of pressure in the termite gut by no means approach those that were used in these experiments, their potential effects cannot be entirely ignored.

Cytoplasmic protuberances similar to that shown in plate 7, figure 26, are commonplace in sections as well as in smears, but the specimen shown is extraordinary because the protuberance is occupied by a loop of the recurvent portion of the axostyle. The protuberance is not an artifact, but has existed long enough to permit the growth of the fibers. It is unlikely that the protuberance was produced by the fiber, because fiber formation is found in only a small fraction of the total number of protuberances. Other instances are shown in plate 3, figure 2, and plate 13, figure 87. In the rostellum the axo-

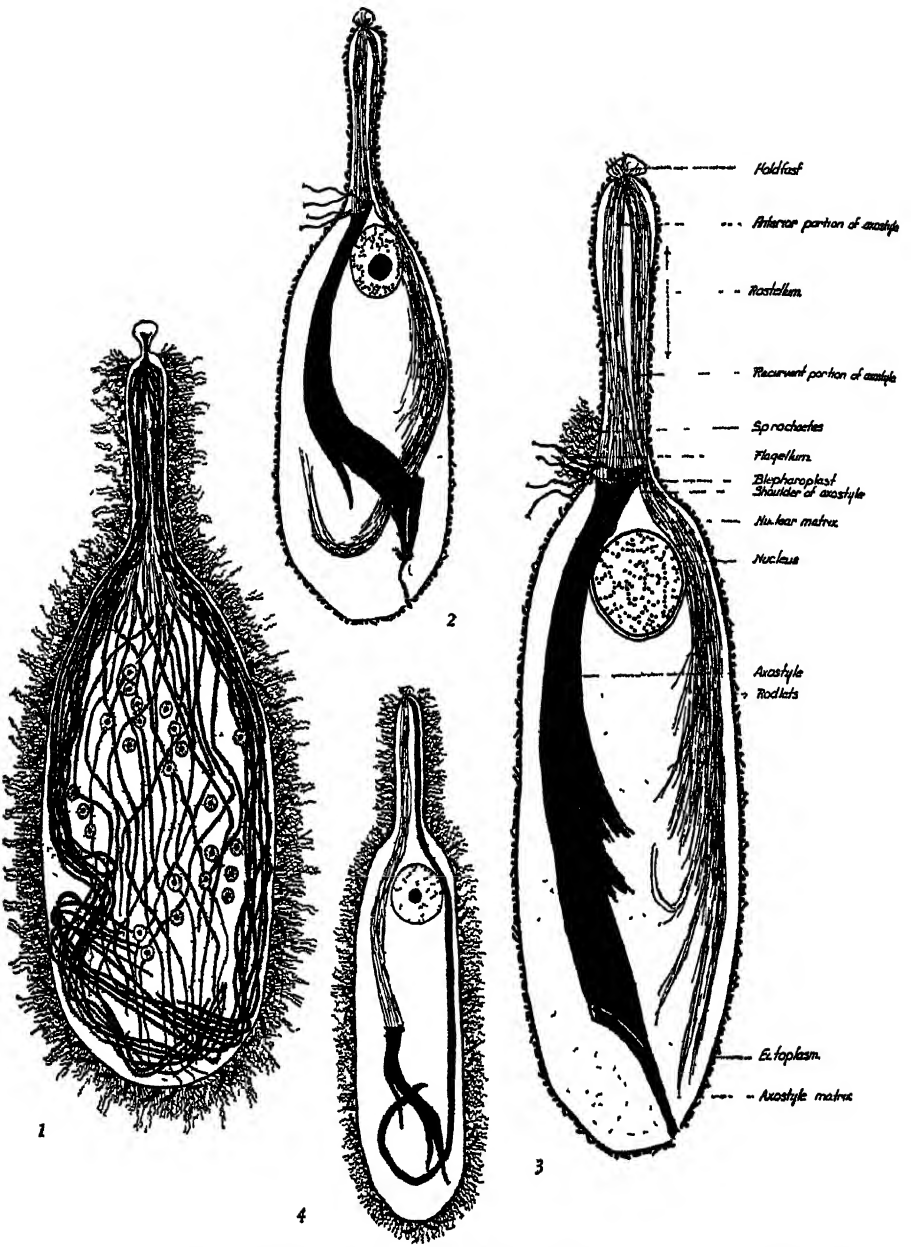


Fig. A. Representatives of the Oxymonadinae showing the diversity in their configuration. 1. *Barroella coronaria*. 2. *Oxymonas megakaryosoma*. 3. *Oxymonas grandis*. 4. *Oxymonas notabilis*. (830 x the median.)

style fibers grow outward, then return; and in the protuberances the same behavior is evident, an outgrowth and a return. A protuberance presumably might result from either a slow, persistent pressure, or a sudden, violent crushing. In the former instance, the cytoplasm within the protuberance could find its new position by slow displacement. In the latter, there would probably be streaming. Possibly the presence or absence of streaming within the protuberance determines the presence or absence of fibers.

In general, my observations on the axostyle agree with Cleveland's report. The shoulder of the axostyle is broadened and trough-shaped, so that both of its anterior corners lie on the same side of the body, at the base of the rostellum, with the edge of the axostyle forming a semicircle between them. The major portion of the axostyle extends excentrically throughout the full length of the body from the shoulder at the base of the rostellum and is composed of closely appressed fibers, some of which fray moderately and irregularly in the posterior half. Usually the posterior edge of the major portion of the axostyle is rolled like a scroll (text fig. A, fig. 3; pl. 8, fig. 33). The posterior end of the thick scroll is drawn out until it forms the slender, distal tip of the axostyle and projects very slightly beyond the posterior boundary of the body. The structure as a whole resembles a scimitar. One blepharoplast is set closely against one corner of the shoulder of the axostyle and the other is attached by a short fiber to the opposite corner, so that the blepharoplasts also tend to lie on the same side of the flagellate's body. Each blepharoplast is composed of two rounded granules, and two flagella originate from the more external granule of each pair. Since one pair of flagella passes outward through the spirochaete tuft, they are very difficult to trace. No measurements were made of the length of the flagella. The specimen in plate 5, figure 16, shows that the typical blepharoplast arrangement is achieved very early in the post-telophase period.

My observations confirm Cleveland's (1935) explanation of the variation in staining reaction between the different portions of the axostyle. The degree of compactness of the paralleling fibrils, of which the various parts are composed, determines the intensity of the staining The regions which stain most deeply are retained the longest during the degeneration which accompanies mitosis. I have divided that section of the axostyle which Cleveland called the "non-staining portion" into two parts, the "anterior portion" and the "recurvent portion."

The anterior portion of the axostyle consists of delicately staining fibrils which pass from the shoulder of the axostyle anteriorly to the holdfast or its anlage. In *O. grandis* the recurvent portion stains delicately and consists of those fibrils that extend backward into the body from the holdfast or its anlage. The fibrils of both the anterior and the recurvent portions are apparently continuous with one another and with those from the major portion of the axostyle. Cleveland described the recurvent portion as being more regular than I have found it( text fig. A, fig 3). Sometimes it was seen as two somewhat frayed ribbons, and sometimes as a broad, almost fan-shaped arrangement of loose fibers.

Where the anterior portion of the axostyle doubles backward at the tip of the rostellum to become the recurvent portion, there are a few fibrils that continue their anterior direction beyond a small deeply stained nodule at the point where they leave their fellows and form the holdfast. The continuity of the fibrils of the holdfast and of the rostellum is established by an occasional less compact agglomeration of fibers than that which produces the nodule. A slight amount of cytoplasm clings to the fibrils, they branch, and the branchlets spread over the chitinous intima of the gut epithelium among the spirochaetes bordering it in much the same fashion as rootlets insert themselves in interstices of rock and make anchorage (pl. 6, fig. 19). When *O. grandis* is torn from the gut, as it may be in smears, the slight amount of cytoplasm that accompanied the fibril seems to contract and round up into more or less complex bulbous structures like those shown by Cleveland (1935) in his text figure A, and by my text figure A, figure 3. If such a soft spheroid of cytoplasm were telescoped backward over a fibrous core, it would produce exactly what Kirby pictured in *Microrhopalodina multinucleata* (pl. 23, fig. 24, 1928). Numerous specimens have been found in which the tips of the rostellum represent intermediate stages between the cytoplasmic spheroids pictured by Cleveland (1935), the knoblike structure by Kirby (1928), and the slightly concave surface, by Kofoid and Swezy (1926) and Connell (1930). The variation in appearance is dependent upon how much of the holdfast was destroyed when the animal was detached from the gut. Some damage is inevitable with animals in smears, but only the terminal fibers are lacking in the drawings presented by Kirby (1928) and by Cleveland (1935).

Kofoid and Swezy (1926) pictured the attachment of *Microrhopalodina multinucleata* by means of a "cup shaped expansion" of the tip of the rostellum to the surface of the epithelium in sections from the intestine of the termite. Kirby's plate 22, figure 10, of *M. multinucleata* (1928) gives a more satisfactory representation of the usual relationship of the rostellar tip of oxymonads to the chitinous intima of the intestine. Duboscq and Grassé (1934) stated that more intense staining was the only modification shown by the rostellar fibers at the point of attachment, and their text figure IV, figure 1, gives no more detail than was found in the earlier reports. They seem to have overlooked the implications of a fibrous attachment indicated in their text figure IV, figure 2; but the appearance of this fibrous attachment more nearly agrees with my observations than do any of the others. Previous reports, however, have truly presented the appearance of the attachment mechanism as it is usually found, and even in so relatively large an animal as *O. grandis,* specimens that are favorable for the recognition of the additional fibrous detail pictured in plate 6, figure 19, are not commonplace.

The term "nuclear matrix" has been substituted for Cleveland's phrase "nuclear sleeve," because a matrix may be defined as an "enveloping element within which something originates, takes form, or develops," and this is a better description of the apparent function and amorphous condition which the organelle assumes during mitosis. In the interkinetic period it is a membranous, sacklike organelle extending posteriorly from the shoulder of the

axostyle (text fig. A, fig. 3) and encircling the nucleus too closely to be visible in many instances. However, it may readily be seen in plate 5, figure 18, and plate 8, figure 34. Its substance is more durable than that of the unmodified cytoplasm of the body, for in specimens where that has been destroyed, the matrix persists and maintains the attachment of the nucleus to the axostyle shoulder.

The nucleus is slightly ovoid, has a well-defined membrane, and in the interkinetic period is situated just posterior to the axostyle shoulder. The chromatin is disposed in discrete granules of somewhat varying size in a reticulum composed of irregular strands of a delicately staining matrix substance (text fig. A, fig. 3). Sometimes the strands of granules form clumps (pl. 5, fig. 18) and sometimes a relatively clear, narrow band separates the central area that is occupied by the loose chromatin reticulum from a slender ring of fine granules, lying just within the nuclear membrane. No centrosomes were observed, and there is no karyosome. In late reorganization stages, however, a karyosome is present (pl. 7, figs. 23, 25, 28) and is often associated with an axostyle the immaturity of which is indicated by the arrowhead shape of its tip (Kirby, 1928; Cross, 1941).

The ectoplasm is usually sharply differentiated as a narrow, clear layer. The endoplasm is finely granular and ordinarily contains wood fragments and spherules that range in color from copper to yellow and are surrounded by vacuoles. The spherules persist after the body has been completely destroyed and are frequently present in the rostellum, which is contrary to Connell's (1930) report. It seems wiser to assume, as Janicki (1915) and Kirby (1928) have, that these structures are metabolic products formed from ingested wood. Zeliff (1930*a*) used the term "chromidia" for those spherules that stain dark with haematoxylin, and Connell (1930) called all of them volutin on the basis of nonspecific staining reactions. Zeliff failed to report any tests supporting his terminology, and my tests (p. 70) did not substantiate either Zeliff's or Connell's claims. Usually if the spherules are lacking the axostyle is much frayed. Contrary to Connell's report, their absence is not correlated with the absence of mitosis.

The pellicular symbionts consist of rodlets arranged in a brush-stroke pattern and spaced rather regularly over the entire surface of the body, including the rostellum, and a tuft of spirochaetes attached to one side of the rostellum just above the shoulder of the axostyle (text fig. A, fig. 3). The position of the latter is even more specific than Kirby (1941*a*) indicated, since the spirochaetes always occur in the region occupied by one pair of the flagella and on the opposite side of the axostyle from that on which the nucleus is situated. Possibly the localization is a consequence of the fact that the region is more sheltered from the periodic changes of mitosis; or possibly it occurs because of a chemotropism, for this side of the rostellum is in much closer association with the intensely basophil shoulder of the axostyle. The spirochaetes are absent occasionally. Perhaps they are dislodged or are obscured beneath the rostellum. They are present on both parts of the dividing rostellum shown in plate 6, figure 20. Occasionally, specimens of *O. grandis* had numerous

colonies of *Sphaerita* in the cytoplasm (pl. 7, fig. 31). Plate 5, figure 17, shows an *Oxymonas* parasitized by a short plump rod. The nucleus has been crowded posteriorly and the axostyle is very broad and pale. Several animals were seen in which similar rods had localized in smaller numbers at the base of a rostellum. Whether the granular appearance of the nucleus has been caused by the parasites' actual invasion of the nucleus or whether it resulted from the hypertrophy of the chromophil substance could not be determined; and it was equally impossible to discover whether the clear circle surrounding the chromophil mass was inside the nuclear membrane, or whether it was outside and represented an atypical expansion of the nuclear matrix.

### Oxymonas megakaryosoma sp. nov.[2]

*Oxymonas megakaryosoma* is a uninucleate species of the Oxymonadinae that occurs in the intestine of *Glyptotermes* sp. nov. from Uganda. Trinucleate specimens (pl. 10, fig. 54) were recorded a number of times and a four-nucleate animal is shown in plate 8, figure 38. *O. grandis* is the only other oxymonad that has been reported with an axostyle that is at all similar to that of *O. megakaryosoma,* but the presence of a large oval karyosome in the latter and its absence from the interkinetic period in the former separate the two species sharply. The facts pertaining to the life cycle and the correlated host relationships in *O. grandis,* and to the morphological modifications which resulted therefrom, are equally applicable to *O. megakaryosoma* (pp. 71–74).

The broad, fibrous composition of the major part of the axostyle which extends excentrically from the rostellum throughout the length of the body and frays moderately and irregularly in the posterior half, is very like that organelle in *O. grandis;* but it appears less rigid and slightly sinuous because it is a little longer than the body. The anteriorly situated, trough-shaped shoulder and its association with the blepharoplast-flagella complex is exactly the same as that of *O. grandis* (pl. 5, fig. 18; pl. 9, fig. 39). The posterior edge is narrower, but it is also rolled into a scroll, one end of which is drawn out and terminates in a heavy fiber which forms the posterior tip of the axostyle and extends slightly beyond the posterior boundary of the body. A cufflike expansion encircles the junction of the scroll and the fiber. The anterior and recurvent portions of the axostyle duplicate the appearance of these structures in *O. grandis.* A specimen is shown, however, in which the rostellum is unusually broad (pl. 9, fig. 43) and which demonstrates why the fibers of the recurvent portion of the axostyle were described as cytoplasmic fibers previous to Cleveland's (1935) report of their origin from, and their continuity with, the anterior portion of the axostyle in *O. grandis.* Because it is smaller, studies of the holdfast were slightly less definite than in the last-named species; but they completely confirmed the findings pictured in plate 6, figure 19. No measurements of the length of the flagella were made.

The nuclear matrix encircles the nucleus as it does in *O. grandis,* but its insertion in the axostyle is anterior to the shoulder and there is a tendency to form accessory fibrils (pl. 9, figs. 39, 40). In one specimen (pl. 9, fig. 40) a

[2] See below, pp. 112–113, for diagnosis and taxonomic position.

rhizoplast was seen extending from the axostyle to one of a pair of prominent granules in, or on, the anterior surface of the nucleus. The nucleus is ovoid and its membrane is delicate. The chromatin granules vary somewhat in size and the reticular strands in which they are embedded frequently appear in short festoons with delicate strands crossing the halo and joining the karyosome (pl. 9, figs. 39, 40). In some specimens both the nuclear membrane and the chromatin granules are so pale that the nucleus is not readily distinguishable, because under such circumstances there is a superficial similarity between the appearance of the karyosome and the darker-staining cytoplasmic spherules.

Since the karyosome is formed by an agglomeration of granules, whether it appears homogeneous or whether it appears to contain a granule, depends upon the compactness of its granular components. The degree of compactness also determines whether the boundary of the karyosome is smooth or irregular. The presence of more than one "karyosome" results from the series of divisions which precedes degeneration of that organelle in prophase.

The division of a clear, narrow band of ectoplasm from the granular endoplasm, and the presence of cytoplasmic spherules and wood particles, is the same as was described for *O. grandis*. The excessive fraying of the axostyle which was often correlated with the absence of spherules occurs in both species, but it is pictured only in *O. megakaryosoma* (pl. 9, fig. 44; pl. 10, fig. 55). The fraying was not limited to animals undergoing division, but specimens in kinesis were chosen for illustration because Connell (1930) had stated that division did not occur if the cytoplasmic spherules were absent.

The rodlets which constitute the pellicular symbionts (text fig. A, fig. 2) are similar in appearance to those of *O. grandis*. Because their attachment is more frequently by one end than by the side, and because there is a greater tendency to chain formation, *O. megakaryosoma* appears slightly more shaggy.

### Oxymonas notabilis sp. nov.[8]

Although *Oxymonas notabilis* is normally a uninucleate animal, binucleate animals are frequent and even six-nucleate specimens are not rare. Its host is *Neotermes howa* from Madagascar. The facts pertaining to the life cycle and the correlated host relationships in *O. grandis* and to the morphological modifications which resulted therefrom, are also applicable to *O. notabilis* (pp. 71–74).

In the immature stages of *O. notabilis*, the shoulder of the axostyle is found, as is usual in the group, at the base of the rostellum in association with two blepharoplasts, each of which is composed of two granules, and in each of which the distal granule supports two flagella (pl. 13, fig. 88). However, in the mature animal, no evidence of either blepharoplasts or flagella was found, although a diligent search was made; and the axostyle shoulder is situated excentrically near the middle of the body. The major portion of the axostyle is composed of closely appressed paralleling fibers extending posteriorly from the relatively straight and narrow anterior edge that forms the

[8] See below, p. 113, for diagnosis and taxonomic position.

shoulder. A flattened, pennant-shaped organelle is formed with a tapering, pointed posterior tip (pl. 13, fig. 87) or with double tips like a swallow's tail (text fig. A, fig. 4). The tips terminate freely in the posterior cytoplasm, near, but not ordinarily in contact with, the posterior boundary of the body. The anterior portion of the axostyle consists of delicately staining fibers that extend from the shoulder to the apex of the rostellum. The recurvent portion is a deeply stained, compact, fibrous, ribbonlike structure, extending posteriorly from the apex of the rostellum into the posterior cytoplasm of the body, where it turns anteriorly again. The recurvent portion of the axostyle is much longer than the body, but always lies within the cytoplasm and never passes beyond the pellicle. Loops of the recurvent portion often occupy cytoplasmic protuberances which occur commonly in *O. notabilis* in spite of its smaller size (pl. 13, fig. 87). The recurvent portion of the axostyle is a part of Cleveland's "non-staining" portion of the axostyle; but in *O. notabilis,* the intensity with which the recurvent portion stains made Cleveland's term completely inappropriate. The details of the holdfast in *O. notabilis* could not be distinguished because of its smaller size; but since the larger structures faithfully duplicated those seen in *O. grandis* and in *O. megakaryosoma,* it is probable that the terminal holdfast fibrils are the same.

A suggestion of a nuclear matrix was seen in the specimens represented in plate 13, figures 82 and 88; but no evidence of it was found in fully developed animals. This was unfortunate, for the modification necessitated by the posterior migration of the axostyle shoulder would have been particularly interesting. In *Oxymonas grandis* the nuclear sleeve was inserted in the axostyle at the level of the shoulder (pl. 5, fig. 18); in *O. megakaryosoma* it was inserted slightly above the shoulder (pl. 9, fig. 40). In *O. notabilis* the insertion occurs possibly at a considerable distance anterior to the shoulder and this permits the retraction of the axostyle shoulder without a concomitant change from the customary position of the nucleus.

The nucleus is broadly ovoid. Fine chromatin granules lie just beneath and close against the thin but well-defined nuclear membrane. Other, slightly larger, granules are embedded in a loose, delicate, reticular matrix. Where the strands of the reticulum cross one another, small clumps of granules often occur. The karyosome is round and excentric posteriorly and the halo which surrounds it is narrow. A few delicate strands of the reticulum cross the halo and join the karyosome.

The clear, narrow band of ectoplasm found in *O. grandis* and *O. megakaryosoma* is lacking in *O. notabilis* but the endoplasm is similarly granular and contains wood particles and cytoplasmic spherules. It is interesting to notice that the latter are smaller in smaller animals and that this is true within as well as between species ( pl. 7, figs. 23, 26, 30; pl. 9, fig. 47; pl. 10, fig. 54; pl. 13, figs. 83, 87).

The pellicular symbionts, consisting of long spirochaetes and long curved rods which are attached by their ends, completely cover the body (text fig. A, fig. 4). Spore formation in the rods was suspected because frequently they appeared more bulbous and more deeply stained at the attached end.

BARROELLA CORONARIA SP. NOV.[4]

*Barroella coronaria* is a multinucleate oxymonad that is found in the intestine of *Neotermes howa* var. *mauritiana* from Mauritius. The characteristics which distinguish *B. coronaria* from *B. zeteki,* the only other member of the genus, are given with the report on the latter species (p. 122). The facts pertaining to the life cycle and the correlated host relationships in *O. grandis* and to the morphological modifications which resulted therefrom, are also applicable to *B. coronaria* (pp. 71–74). No other member of the Oxymonadinae has shown as extensive variations as this species. Variability in the size of the body and of the rostellum is well demonstrated in plate 14, figures 91 and 92, and in plate 15, figure 108. Cyclical changes in bodily appearance are shown in plate 15, figures 102 to 105. The name *coronaria* describes the multiple crown-making stage illustrated in plate 15, figure 108.

Uninucleate oxymonads are present in the same host *with B. coronaria* and their nuclei are not unlike those of certain immature stages of the latter. However, no specimens were seen in which the uninucleate form originated from the multinucleate animals, and an insufficient number of division stages of the uninucleate form was found to determine certainly what the interkinetic appearance of the nucleus might be. Moreover, in sections from the intestine of the termite, there was always a marked segregation of uninucleate and multinucleate animals, which is contrary to the expected condition if the former is actually a division product of the latter.

The major portion of each axostyle is slender and extends posteriorly from its shoulder either to the posterior boundary of the body or for a slightly shorter distance. The anterior portion consists of delicately stained fibers, but the recurvent portion is an intensely stained, ribbonlike structure which is much contorted because it lies entirely within the body in spite of its length. In the latter respect, it is similar to *O. notabilis.* However, the shoulder of the axostyle is tapered slightly and not broadened, as is true of the latter species, and it is not retracted to the central region of the body but approximates the position near the base of the rostellum that is found in all of the other oxymonads, excepting *O. notabilis.* Instead of a nodule at the apex of the rostellum, there is a deeply stained cylindrical extension of the rostellar fibers which is about twice as broad as the ribbonlike recurvent portion of one of the axostyles. Although the studies of the holdfast were less satisfactory than were obtained for *O. grandis* and for *O. megakaryosoma,* the evidence implies that attachment is made in the same manner by fibrils extending from the cylindrical projection, which is homologous with the nodule of the uninucleate species.

No blepharoplasts nor flagella were observed in the mature animal, but in plate 14, figure 96, are shown numerous very young axostyles in which the blepharoplast-flagella complex was distinct and which illustrated the high degree of flagellar mutilation that occurred. Plate 14, figure 97, shows two axostyles, one with a total of two flagella and the other with only one. The

[4] See below, p. 123, for diagnosis and taxonomic position.

irregularities in flagellar number occurred indiscriminately on either the proximal or the distal blepharoplast, and plate 14, figure 100, probably represents the unmutilated state. One blepharoplast is in close association with the shoulder of the axostyle and the other is joined to it by a short, slender interblepharoplast fiber. Each blepharoplast is composed of two closely appressed spherical granules, and two flagella originate from each of the more distal granules of the pair. Earlier mutilation probably accounts for the presence of only three flagella in plate 15, figure 106.

In another immature specimen similar to that shown in plate 15, figure 103, flagella were seen that were one and a half times the length of the body. Because the flagella and blepharoplasts were very much crowded in the corona formation, it was not possible to determine whether each axostyle was associated with two blepharoplasts and four flagella, but two flagella were found originating quite consistently from each blepharoplast.

In the interkinetic period, the nuclei are scattered throughout the body of *B. coronaria*. Usually, they are without any appearance of order, but occasionally they have been observed in longitudinal paralleling lines of five or six nuclei in each. This seems to have resulted from the chance collection of the nuclei between two adjacent axostyles.

In *O. notabilis*, the nucleus lost its close association with the axostyle shoulder and there was no evidence of a nuclear matrix in the interkinetic stage. The units composing the multinucleate *B. coronaria* exhibit this same lack of a matrix that is correlated again with the disassociation of the nuclei and their respective axostyle shoulders. The nuclear matrix, however, is readily distinguished in association with a very young nucleus (pl. 15, fig. 106) which is from the late division period illustrated in plate 15, figure 108. The interkinetic nucleus is spheroidal in shape. The membrane is definite and the chromatin granules are relatively large and distributed rather uniformly in a fine-meshed reticulum composed of a delicately staining matrix substance. The karyosome is round, and surrounded by a halo which is crossed occasionally by a delicate strand of the matrix reticulum.

There is no division of the cytoplasm into a clear ectoplasm and a granular endoplasm in *Barroella coronaria*, a characteristic in which it again resembles *O. notabilis*. The cytoplasm contains wood particles, and the spherules are usually very small. They have been omitted in the illustrations for the sake of clarity. Frequently, the anterior third of the body is relatively clear in appearance and although the nuclei are definitely in interkinesis, the axostyles have assumed the condition shown in plate 14, figure 92.

The pellicular symbionts are the largest that have been recorded in this paper, and they cover the animal thickly, and completely (text fig. A, fig. 1). The rods are similar to those seen attached to *O. notabilis* in their tendency to be bulbous and to stain more intensely at their proximal ends. They were not found on the uninucleate *Oxymonas* that occurs in the same host with *B. coronaria*, and the spirochaetes covering the former are thicker, and shorter than the longest ones found on the latter.

## SPECIAL STUDIES OF REPRODUCTION

### Historical Account

Although morphological diversification and variation are extreme among the Oxymonadinae, the pattern of mitosis among them is fairly uniform and many of the differences in kinetic behavior that have been reported previously are actually only differences in interpretation. Janicki (1915) reported that the new axostyles originated from the old intranuclear spindle in *Oxymonas granulosa;* but in spite of their diagrammatic quality, his illustrations demonstrate the fallacy of his statement. Both Zeliff (1930*a*) and Connell (1930) described the intranuclear spindle as developing from the karyosome. Unless the absence of a karyosome whenever the spindle is present, or the converse, is accepted as proof, Zeliff's illustrations do not substantiate his report; and the elongating karyosome shown by Connell is the initial stage of repetitious divisions which result in the complete disintegration of that organelle instead of the formation of a barlike centrodesmose, as he stated. My description of a nuclear matrix involves new factors, but Kirby (1928, pl. 24, fig. 43) pictured it; Cleveland (1934, pl. 60, figs. 442–445) pictured it and called it a halo; Connell (1930) recognized it as the "clear area"; and Janicki (1915) illustrated it and called it a "transparenten Plasmaportion."

Janicki's (1915) account of kinesis was handicapped by an insufficient number of specimens. In his report of mitosis in *Microrhopalodina multinucleata,* Kirby (1928) recognized, for the first time, posterior migration of the nucleus during the prophase, described the structure of the spindle, and figured but did not describe the metaphase girdle of chromatin. Zeliff (1930*a*) both reported and figured the latter, but unfortunately his numerous illustrations are very small. Connell (1930) suggested a correlation between the origin of the neuromotor system and the "clear, undifferentiated substance," and reported that mitotic figures of *O. dimorpha* were found only in smears from the intestine during the first few days following ecdysis in the termite. Cleveland's (1935) description of the achromatic figure in *O. grandis* discredited the idea that the karyosome produced the spindle as supposed by Janicki (1915), Zeliff (1930*a*), and Connell (1930). The application of his report of the origin of the recurvent portion of the axostyle to the earlier accounts of retractor fibers (Kofoid and Swezy, 1926*a*) and cytoplasmic fibers (Kirby, 1928; Connell, 1930; Lewis, 1933) has been discussed earlier in this paper.

### Nuclear Migration

My observations of mitosis in *O. grandis, O. megakaryosoma, O. notabilis,* and *Barroella coronaria* have confirmed my description (1941) of an anterior posttelophase migration of the nucleus preceding plasmotomy in *O. minor.* In conjunction with Kirby's report (1928) of a posterior prophase migration of the nucleus it establishes the presence of an orbital nuclear migration concomitant with intranuclear cyclical kinetic changes. An attempt to determine the correlation of the stages of this cycle within a cycle has resulted in the following briefly outlined conclusions.

In *Barroella coronaria,* the morphological unit of one nucleus and one axostyle has been used to facilitate comparison of this multinucleate flagellate with the uninucleate oxymonads. The use of the unit is altogether feasible because mitosis is synchronized in all the nuclei of multinucleate oxymonads.

In the prophase the nucleus migrates posteriorly (pl. 3, figs. 2, 6, 8; pl. 9, fig. 47; pl. 11, figs. 59, 62; pl. 14, fig. 92) (Kirby, 1928; Zeliff, 1930*a*; Connell, 1930; Cleveland, 1935), and usually this migration continues through the metaphase with the long axis of the nucleus paralleling the long axis of the body. In the anaphase, there is a swift shifting in orientation that leaves the nucleus in the extreme posterior part of the body with the long axes perpendicular. In very small animals, the long axis of the nucleus may remain parallel to the long axis of the body. The telophase occurs in the same position as the anaphase.

The post-telophase anterior migration of the nucleus preceding plasmotomy reported for *O. minor* (Cross, 1941) has been amply verified (pl. 6, figs. 20, 22; pl. 8, fig. 33; pl. 10, fig. 55; pl. 13, figs. 82, 83, 85, 89) and is essential to an understanding of late mitotic figures in this flagellate subfamily. With its interpolation into the reproduction pattern, division specimens such as text figure C, figure 4 in Kofoid and Swezy, 1926*b*, and plate 24, figure 33 in Kirby, 1928, need not be accepted as evidence of fission unaccompanied by mitosis. Cleveland's (1935) figure 6, in text figure B, no longer implies transverse division of the body, contrary to flagellate behavior, but becomes an illustration of anteriorly migrating nuclei which must undergo the successive stages represented in plate 6, figure 22; and plate 7, figures 31 and 30.

Even in the complex multinucleate species *Barroella coronaria,* the concept of nuclear migration proved valid. Prophase migration of the nucleus is often obscured because the nuclei are already situated posteriorly during interkinesis. Anterior post-telophase migration of the nucleus appears in a modified form, indicated at first by the polarization of all of the nuclei and the axostyles toward the anterior part of the body (pl. 15, fig. 109; pl. 16, fig. 115). This orientation is an exact duplication of the initial step in the anterior nuclear migration in uninucleate animals. Instead of a centralization of nuclei at the extreme anterior end of the body, which is manifestly impossible when large numbers of nuclei are involved, group localizations occur at numerous loci throughout the animal (pl. 15, fig. 108). The subsequent steps of repetitious multiple division are easily and successively traced in plate 15, figures 102, 105, 104, 103, and terminate in a *Microrhopalodina*-like stage.

Theoretically, this process does not exclude the possibility of uninucleate animals deriving from multinucleate forms. Sometimes, cytoplasmic division instead of appearing as a budding mass (pl. 15, fig. 105) occurs with the "buds" arranged linearly, in a long, drawn-out chain. Such behavior would seem to be more favorable to the production of uninucleate offspring, but since no specimens were found illustrating their origin, and since there were nuclear differences as well, division in *B. coronaria* is not reported as terminating in a uninucleate state.

Connell's hypothesis of the production of multinucleate from uninucleate

animals by means of delayed plasmotomy is supported by the experimental production of binucleate specimens of *Amoeba proteus* (Chalkley, 1935) in which cytokinesis was entirely eliminated whenever it was prevented until after the daughter nuclei had passed a certain stage in reorganization. The presence of anterior post-telophase migration of the nuclei preceding plasmotomy increased the plausibility of Connell's postulate, because otherwise it was necessary to assume some special method of returning the daughter nuclei and their axostyles to their normal interkinetic position from the posterior region of the body where they are scattered at the close of division.

### Extranuclear Organelles

Ordinarily, during the prophase the old axostyle degenerates progressively but the fibrils of the holdfast are retained. Perhaps its isolated position protects it from the fiber-destroying physiological changes within the body at this period and permits the holdfast to maintain the sessile condition of the animal through much, or all, of the division process.

The nuclear matrix loses its connection with the axostyle and usually becomes a more or less amorphous, clear area which accompanies the nucleus in its posterior migration (pl. 3, fig. 2; pl. 9, fig. 47; pl. 11, figs. 58, 60, 61, 62). Probably the sharply defined, external capsule surrounding the nucleus in plate 3, figures 5 and 6, and the definitely outlined clear area extending from the rostellar region to encircle the posteriorly situated nucleus in plate 11, figures 57 and 59, can be explained as a retention of the earlier membranous condition of the matrix.

During kinesis the amorphous nuclear matrix continues to surround the nucleus, and since young axostyles have not been found originating or developing except along the border between it and the granular cytoplasm, the closeness of association between the daughter axostyle and the nucleus is always dependent upon the elastic volume of the surrounding matrix.

The separation of the nuclei from their more rapidly migrating daughter axostyles is only apparent, not actual (pl. 5, fig. 15), and the amorphous nuclear matrix supplies the connection which Cleveland (1935) did not find but argued must be present continuously. The matrix is not only divided between the daughter nuclei, but is often branched to form an axostyle matrix as well (pl. 4, fig. 14; pl. 10, fig. 51; pl. 13, figs. 81, 82, 85, 88, 89, 90). If the readiness with which this substance produces a membrane for the interkinetic nuclear matrix and its association with the fibrous production of young axostyles are recalled and considered in relation to the configuration of the axostyle matrices in plate 13, figures 88 and 90, the "axostyle sleeve" of Kofoid and Swezy (1926*a*) may be plausibly explained as the casual retention during early interkinesis of a voluminous and potentially fibrous or membranous nuclear matrix, and it may be more appropriately called a matrix sleeve.

In only one instance has an axostyle been found actually within a sleeve. Instead, the inner position is often held by the nucleus, and the axostyle lies along the periphery of the matrix which maintains the same relative positions

that the matrix and the axostyle held during the anaphase. In an undescribed multinucleate oxymonad in *Kalotermes tuberculatus* from Australia, all of the axostyles were encircled by a single, fibrous matrix sleeve as though the latter had been produced by the fusion of all their individual matrices during their mutually amorphous condition in the telophase. The matrix is frequently seen in the motile period of *Oxymonas* where Connell (1930) described its function as being that of protection to the neuromotor system; he differentiated it from the axostyle sleeve reported by Kofoid and Swezy (1926*a*) because of its dissimilar function and structure. Actually, it seems that Kofoid and Swezy based their description on a later and somewhat fortuitously modified period of the same matrix substance. There can be no doubt that the nuclear matrix anchors the nucleus to the axostyle during interkinesis. Possibly, as Connell stated, it also protects the blepharoplast-flagella-axostyle complex during the motile period, but none of my observations have supported the idea that the matrix functions in the active extension and retraction of the rostellum as Kofoid and Swezy (1926) reported it.

The earliest evidence of a new axostyle in *O. grandis* is the granule attached by a fibril to one pole of a prophase nucleus (pl. 3, fig. 7). In plate 9, figures 47 and 48 show longer fibrous extensions across the nuclear matrix from the spindle poles. There are two pairs of granules that are somewhat larger than the neighboring cytoplasmic granules just beyond the spindle poles in plate 11, figure 65; but these cannot be regarded as anlagen of the new axostyles. Well-differentiated young axostyles are pictured in specimens in the metaphase and the anaphase (pl. 4, figs. 10, 11; pl. 9, fig. 50; pl. 10, fig. 52; pl. 11, figs. 63, 66, 69), and in many of them a fibrous connection with the spindle poles can be traced. The origin of the new axostyle seems to be at the point where the fibrous extension from the spindle pole touches the peripheral border of the amorphous nuclear matrix. From this point, compact, deep-staining fibers grow along the peripheral border of the matrix to produce the major portion of the axostyle. Less compactly spaced fibers grow in the opposite direction to produce the anterior portion. The nuclear matrix is attached to the axostyle at the anlage of the shoulder, which exists as the line of demarkation between the two parts of the axostyle (pl. 4, fig. 14; pl. 10, figs. 51, 56). At a short distance from the shoulder, the anterior portion doubles back sharply upon itself to produce the recurvent portion of the axostyle (pl. 5, fig. 15; pl. 9, fig. 50; pl. 13, figs. 81, 90). Nodules appear along the fibers at the locus of transposition from the recurvent to the anterior portion of the axostyle and apparently constitute the anlage of the holdfast (pl. 4, fig. 13; pl. 5, figs. 15, 16; pl. 13, fig. 81).

Cleveland (1935) reported that an achromatic figure producing part of the centriole remained inside the nucleus but that another part migrated out from the nucleus and functioned in the production of the axostyle. I have seen no such migration, but there have been many instances where definite fibrils have extended outward from the pole of the nucleus (pl. 3, fig. 7; pl. 9, fig. 48; pl. 10, fig. 52; pl. 11, fig. 63), and their presence certainly implies a definite relationship between the spindle pole and the origin and development of the

axostyle. In the telophase of *O. grandis,* blepharoplasts and flagella may be seen in much the same arrangement as they appear in the interkinetic period, with two blepharoplasts each supporting two flagella. No specimens of *O. megakaryosoma* were found that furnished satisfactory studies, but plate 10, figure 56, shows the lower axostyle with two blepharoplasts situated at the intersection of the borders of the nuclear matrix and the axostyle shoulder. One blepharoplast evidently consists of two granules. It is possible that the granules attached by fibrils to both of the poles of the nucleus in plate 10, figure 52, are blepharoplasts, but no intermediate stages were found and their association with the axostyle shoulder is less close than is to be expected from the interkinetic position of the blepharoplasts. It is barely possible to distinguish two minute blepharoplasts at the tip of one of the axostyles in the telophase specimen of *O. notabilis* (pl. 12, fig. 72). Similar granules may be detected in plate 13, figures 82 and 88, but no flagella were observed at any stage in *O. notabilis.* Telophase nuclei with blepharoplasts and flagella were abundant in plate 14, figure 96, but the flagella were omitted from the illustration for the sake of clarity. Because the axostyle shoulder in *B. coronaria* is very slender, the arrangement of the blepharoplasts appears slightly different from that of *O. grandis* and *O. megakaryosoma,* but it is essentially the same (pl. 14, fig. 100).

Throughout the group, one new axostyle normally develops at each pole of a dividing nucleus (pl. 4; pl. 10; pl. 12; pl. 15, fig. 110) and theoretically should result in an equal number of axostyles and nuclei in interkinesis. Variation from this established pattern requires explanation (pl. 10, fig. 54; pl. 14, fig. 96). The extra axostyles dragging across the isthmus joining the bud to the body in plate 15, figure 102, support the assumption that the connection between the nucleus and its axostyle might be destroyed in the confusion attendant on anterior migration, resulting in subsequent unequal distribution in plasmotomy.

Another explanation must be found, however, for plate 14, figure 96. Either some nuclei have degenerated or there has been an excessive production of axostyles. Since no axostyles have been observed to originate except in juxtaposition with the nuclear matrix near the poles of a spindle, it is imagined that in the event of the disjunction of an axostyle from a daughter nucleus in the crowded mitotic condition, axostyle growth might be provoked anew by the fibrous extension from the spindle where it crosses the periphery of the nuclear matrix.

In plate 12, figures 75, 77, and 78, however, the nuclei are in excess of the axostyles. Binucleate animals are commonplace in *O. notabilis* and figure 77 pictures a formerly binucleate specimen with both of its nuclei in concomitant mitoses. Figures 75 and 78 probably show instances where a uninucleate animal has undergone two swiftly successive mitoses, omitting the anterior migration of the nuclei, and retaining an undivided nuclear matrix. Since the previously formed axostyles already occupy the region where young axostyles originate, the growth of additional organelles does not take place and the second division results in four daughter nuclei with only two axostyles.

Frequently, the daughter axostyles migrate anteriorly more rapidly than do their respective nuclei (pl. 5, fig. 15). The anterior portion of the axostyle functions as a "burrowing" tip and either produces a new rostellar mound for one axostyle, or both axostyles migrate forward into an existing rostellum which splits longitudinally (pl. 6, fig. 20). Kirby (1928) described the splitting of the rostellum in *Microrhopalodina multinucleata* as beginning proximally, but I have observed it beginning either proximally or distally. The axostyles in plate 6, figure 20; plate 7, figure 23; and plate 8, figure 33, all show arrowhead-shaped posterior tips. Since the binucleate animals are evidently young dividing animals, it is reasonable to suppose that the uninucleate specimen has also recently undergone plasmotomy (Kirby, 1928), and throughout my study of the Oxymonadinae, specimens with arrowhead-shaped tips have been avoided for diagnostic purposes because of the implied immaturity.

## Mitosis

In *Oxymonas grandis* a karyosome is found only in recently divided animals (pl. 7, figs. 23, 25) and it disintegrates by a series of divisions before interkinesis (pl. 7, figs. 24, 27, 28, 29). In the remainder of the group studied, the karyosome divides repeatedly during the prophase. Sometimes when the very large karyosome of *O. megakaryosoma* breaks down, the impression is given of four or five karyosomes. In plate 9, figures 40, 45, 46, 42, and 49 illustrate the progressive changes. The dividing karyosome spins out fibers between its parts and often appears as an ill-defined spindle. Its true character becomes apparent when four or five of these imitations of a spindle occur simultaneously. In *O. grandis* the Feulgen reaction indicates definitely that the karyosome is composed of chromatin and that the spindle fibers, which react negatively, are quite dissimilar from the karyosome in their chemical constitution. Connell (1930) reported that the nuclei of *O. dimorpha* reacted negatively to the Feulgen test although the nuclei of other protozoa on the slide were well stained. My results from varying the length of time for hydrolyzing indicate that probably Connell's failure to stain the nuclei of *O. dimorpha* was caused by insufficient exposure to the hydrolyzing agent. Because in other respects the nuclear components of *O. grandis* are similar to those of other oxymonads, it is logical to suppose that the karyosomes in the entire group are composed of chromatin, and consequently the earlier reports of the production of the spindle from the karyosome (Zeliff, 1930*a*; Connell, 1930) are again discredited (Cleveland, 1935). Sometimes the karyosome has been seen simultaneously with the spindle (pl. 9, fig. 45; pl. 11, figs. 61, 62) (Kirby, 1928, pl. 24, fig. 41). Zeliff's (1930*a*) report that the karyosome originates from the remains of the intranuclear spindle is also invalidated by the very different reactions of the two organelles to the Feulgen test.

However, there is evidently a correlation between the disintegration of the intranuclear spindle and the origin of the young karyosome, for their association is close and practically uniform in all of the one hundred and fourteen nuclei in a specimen of *Barroella coronaria* (pl. 16, fig. 112). The details of the agglomeration of the chromatin granules around the fibrous desmose are

shown in plate 16, figure 111. In my earlier studies of uninucleate animals, the value of similar specimens was discounted because the association was attributed to chance alone. In *O. grandis* and *O. megakaryosoma,* which are much larger animals, the granules could be observed to cluster around the exit through the nuclear membrane of the old spindle (pl. 10, fig. 53). Since the karyosome originates from an agglomeration of granules, the presence or absence of a granule within it demonstrates the degree of compactness of the aggregation rather than the presence or absence of a division center (Janicki, 1915; Kofoid and Swezy, 1926*a*).

Changes from the interphase position of the chromatin granules were recognized in the prophase, but no uniform pattern of behavior was discovered among the oxymonads. Zeliff (1930) reported that there are seventeen chromosomes in *Oxymonas pediculosa* from *Rugitermes panamae.* My observations of *O. grandis, O. megakaryosoma, O. notabilis,* and *Barroella coronaria* are in agreement with Cleveland's (1935) account of *O. grandis,* in which it is stated that the chromosomes are numerous, small, irregular granules. They are embedded loosely in discontinuous strands of a lighter staining matrix substance. Paired strands were commonly seen in the prophase, and Zeliff (1930*a*) described a double thread formation as the result of longitudinal splitting of the chromosomes in this period. Very early spindles were occasionally seen in nuclei that were still in the anterior interphase position (pl. 9, figs. 41, 44, 45) as well as in the expected posterior position. In the four species studied, the earliest definite spindles are similar in that they fail to span completely the breadth of the nucleus (pl. 3, figs. 5, 6; pl. 9, figs. 41, 44; pl. 11, figs. 58, 60; pl. 14, figs. 91, 93). In the nucleus of *O. grandis* the chromatin remains in a loose, granular reticulum, but in the other species it is mostly compacted into a somewhat rounded, dark mass which is penetrated by the spindle fibers. In *O. notabilis* and in *Barroella coronaria* the remainder of the nuclear space is filled with minute discrete granules, but in *O. megakaryosoma* the small granules are in a delicate, fragmented reticulum. The spindle in the specimen of *O. notabilis* (pl. 11, fig. 58) is presumably the earliest one that has been figured, and it evidently consists of one or two closely appressed, stain-resistant fibers. Cross sections of the chromatin mass at this period appear as rings (pl. 14, fig. 91), demonstrating the deeply internal position of the first fibers and explaining the difficulty in discovering earlier spindle formation.

Slightly later a cross section through the chromatin mass appears as a broken ring, because the spindle moves to a peripheral position and begins to assume a cylindrical shape. A pair of granules, sharply outlined by a halo, was seen in a prophase nucleus of *O. megakaryosoma* (pl. 9, fig. 46). It is not impossible that these granules are the centrioles that separate and spin out between them a slender fibril. Whether the formation of the spindle is brought about by the meeting, overlapping, and joining of the astral rays as Cleveland (1935) described it, could not be determined, but certainly the intranuclear spindle is not a bar (Connell, 1930, Zeliff, 1930*a*) but is built up of an increasing number of more or less compact paralleling fibers (pl. 3, figs. 3, 4, 7; pl. 9, figs. 41, 45, 48; pl. 11, figs. 58, 60, 61, 65).

In material stained with Heidenhain's haematoxylin those fibers which Cleveland (1935) stated were formed first can be best described as cablelike in the anaphase and telophase periods (pl. 4, figs. 12, 13; pl. 5, figs. 15, 16; pl. 10, figs. 51, 53, 56; pl. 11, fig. 68; pl. 12, figs. 71, 72). In order to differentiate this distinctive-appearing, earliest-formed part of the intranuclear spindle from the remainder, it will be referred to as the cablelike portion of the spindle. Few of Cleveland's (1935) mitotic figures of *O. grandis* show it and its absence is in agreement with my observations on material stained with Delafield's haematoxylin. It is never difficult in specimens that have been stained with Heidenhain's haematoxylin to distinguish the cablelike portion from the remainder of the spindle in the metaphase. During this period in *O. grandis* (pl. 4, figs. 10, 11), the chromatin granules congregate to form an equatorial girdle (Zeliff, 1930*a*; Cross, 1941). In *O. megakaryosoma, O. notabilis,* and *Barroella coronaria* (pl. 10, fig. 52; pl. 11, fig. 66; pl. 16, fig. 114) the equatorial girdle is formed by an expansion of the smaller more compact mass of chromatin that appeared in the prophase. Frequently, the chromatin granules can be distinguished in longitudinally parallel strands that are of the same length as the girdle. Zeliff (1930*a*) described this arrangement of the chromatin in the girdle but he also stated, "The splitting of the chromosomes in the metaphase is apparently in a transverse direction, for the chromosomes seem to be in the form of a continuous thread as they lie across the equator of the spindle." It is difficult to understand how the latter part of his statement indicates transverse splitting of the chromosomes. It seems more probable that an entire strand from each of the paired threads passes to each pole. Such a movement is not apparent during their migration from the equator in the early anaphase, but the reappearance of granular chains that are approximately equal in length to those which appeared in the metaphase girdle and that radiate from the poles in late anaphase and telophase, supports the assumption (pl. 4, fig. 13; pl. 11, figs. 68, 69; pl. 14, figs. 92, 98, 99, 101).

Occasionally, beginning in the metaphase and continuing through the anaphase and the telophase, the cablelike portion of the spindle increases in length and creates (or occupies) a bulge on one side of the nucleus (pl. 4, figs. 12, 13; pl. 10, fig. 51; pl. 12, figs. 72, 73); and in some specimens, in addition to lengthening, torsion of the cablelike structure is also evident. Bělař (1926) and Wenrich (1940) have shown that there is a tendency toward torsion in the dividing nuclei of certain amoebae, but in them the twisting of the elongate nucleus is less striking because it is not accentuated by a cablelike portion of the intranuclear spindle as it is in *Oxymonas.*

Cleveland (1934) has shown specimens of *Eucomonympha, Trichonympha, Urinympha,* and *Barbulanympha* in which the nuclei are asymmetrical and in a similar stage to that shown in *Oxymonas megakaryosoma* (pl. 10, fig. 51); but in contrast to the former species in which the extranuclear spindle spans the lesser curvature of the nucleus, in the oxymonad the cablelike portion of the intranuclear spindle outlines the greater curvature.

Such an extreme bulging of the spindle as is shown in *Oxymonas grandis* (pl. 4, fig. 12) may not always take place, but similar specimens were not

uncommon in serial sections, and indicate that the cablelike structure may serve as an active agent in the division of the nucleoplasm. It is difficult to reconcile such figures with Cleveland's (1934) statement in respect to *Barbulanympha* that the spindle serves as a "stabilizer which holds the two symmetrical halves of the dividing cell together until . . . the proper distribution of the chromosomes has occurred and the required development of the extranuclear organelles has been carried out." If the cablelike structure is not actively engaged in the division of the daughter nuclei, at least it must serve to direct the migration of the nucleoplasm toward the poles; and because of the horseshoe shape of the cablelike portion of the spindle, the resulting daughter nuclei would have their poles advantageously oriented for the post-telophase anterior migration. This migration often proceeds with sufficient swiftness that remnants of the old cablelike portion of the intranuclear spindle are still in existence at its completion (pl. 6, figs. 21, 22; pl. 13, figs. 81, 82, 84, 85, 89, 90).

It is not impossible that division of the nucleoplasm results from a sudden straightening of the bowed, cablelike portion of the spindle. In plate 10, figure 53 indicates that this might take place and that the division may result in daughter nuclei that are very unequal in size. No other excessively long nuclei have been seen. Since the nuclear membrane appears very delicate, as though it had been stretched excessively, either the sacklike extension contracts into a normally rounded daughter nucleus, or the membrane ruptures and the nucleus disintegrates. It is also possible that the extraneous part is pinched off and discarded.

Specimens in the late telophase typically appear very similar in *O. grandis*, *O. megakaryosoma*, *O. notabilis*, and *Barroella coronaria* (pl. 5, fig. 16; pl. 10, fig. 56; pl. 12, fig. 80; pl. 14, fig. 97). The daughter nuclei are attached to either end of the spindle and produce a dumbbell-shaped figure. Examination of figure 80 in plate 12 shows that the poles and a considerable part of the spindle adjacent to them lie inside the nucleus. The chromatin exists in coarse granules that radiate in chains from the poles for about half the length of the nucleus, and a small space is always present between their polar ends and the nuclear membrane.

The agglomeration of chromatin granules around the cablelike structure of the spindle, which produces the karyosome, usually begins in late telophase. The degeneration of the central, extranuclear region of the cablelike portion of the spindle progressively follows. The chromatin granules become smaller and begin to spread into the posterior region of the nucleus as a delicate reticulum (pl. 6, fig. 21; pl. 13, figs. 81, 90). Concomitant with these nuclear changes in the uninucleate species, the anterior post-telophase migration of the daughter nuclei returns them to the usual interkinetic position at the base of the rostellum. A comparable stage of localization in the multinucleate *B. coronaria* results from the modified anterior migration of the daughter nuclei (pl. 15, fig. 108).

Longitudinal division of the body is initiated (pl. 6, fig. 20; pl. 8, fig. 37; pl. 10, fig. 55; pl. 13, fig. 85; pl. 15, figs. 102, 105) and continues (pl. 7, fig. 31)

until the daughter nuclei are situated at opposite ends of the animal (pl. 7, fig. 30; pl. 8, fig. 38; pl. 13, fig. 84; pl. 15, fig. 104). By this time a karyosome has been formed in almost all of the nuclei. Nuclear reorganization in the immature daughter animals (pl. 7, figs. 23, 24, 25, 27, 28; pl. 13, fig. 88; pl. 15, fig. 103) both precedes and follows cytokinesis and consists of a redistribution of the chromatin granules to produce the typical reticulum which surrounds the halo-encircled karyosome of the interphase nucleus. In *O. grandis*, however, a further change takes place and the karyosome disintegrates by successive divisions until an exclusively reticular condition of the granular chromatin is reached.

## REPRODUCTION IN THE SUBFAMILY OXYMONADINAE

On the basis of these studies of *Oxymonas grandis, O. megakaryosoma, O. notabilis,* and *Barroella coronaria,* an outline of mitosis in the Oxymonadinae can be given. In the prophase, the nucleus migrates posteriorly, the axostyle degenerates, and, if it is present, the karyosome disintegrates. The presence of more than one karyosome is a consequence of successive divisions which culminate in its complete disintegration. The spindle originates as a few fibrils that are much shorter than the breadth of the nucleus. The fibrils increase in length and in number and the group becomes more compact to form a bulging, cylindrical, achromatic figure in which the first-formed fibers are distinguished as a heavy, cablelike structure when specimens that are in, or have passed, the metaphase are stained with Heidenhain's haematoxylin. Chromosomal fibers are numerous.

The chromatin granules are assembled in an equatorial girdle which is composed of parallel granular strands. New axostyles take origin where fibrils from the spindle poles cross the periphery of the nuclear matrix. In the anaphase, the chromatin granules pass to the poles, their mass radiating in compact strands for about half the length of the nucleus. The cablelike portion of the intranuclear spindle occupies the greater curvature of an asymmetric nucleus, increases in length, and often undergoes torsion. The axostyles continue their growth, differentiate into major, anterior, and recurvent portions, maintain their connection with the nucleus by means of the amorphous nuclear matrix, and develop the anlage of the holdfast.

In the telophase, fission of the nucleus produces two daughter nuclei, widely separated at either end of the cablelike portion of the spindle. New blepharoplasts and flagella appear. Preceding plasmotomy a post-telophase anterior migration of the nuclei and the axostyles occurs in uninucleate oxymonads and results in the localization of the daughter nuclei in the usual interkinetic position at the base of the rostellum. In multinucleate genera a modification of this behavior results in numerous centers for localization of the daughter nuclei throughout the body.

The middle region of the cablelike portion of the spindle disintegrates first. A karyosome is formed from chromatin granules which agglomerate along the old cablelike portion of the spindle. Reorganization of the remaining chromatin granules in the nucleus both precedes and follows cytokinesis.

In *Oxymonas* the anterior portions of the young axostyles either invade lengthy, preëxisting rostella or produce low mounds along the anterior body wall. In the multinucleate genera, the young rostella appear as low mounds extending beyond the body at the localization centers. The existence of an arrowhead-shaped posterior tip on an axostyle is correlated with immaturity.

Plasmotomy is longitudinal and may result in either motile or sessile animals. Splitting of the rostellum may originate either distally or proximally. In sessile animals the holdfast may persist throughout division. Mitosis occurs in either the small motile or the large sessile oxymonads and is independent of the presence or absence of cytoplasmic spherules.

## DESCRIPTIVE AND SYSTEMATIC ACCOUNT

### Family Pyrsonymphidae Grassi

Pyrsonymphidae Grassi, 1892, R. C. Accad. Lincei, (5) vol. 1, 1 sem.: 36; Grassi and Foà, 1911, Rend. R. Accad. Lincei, (5) vol. 20, 1 sem.: 726; Buscaglioni and Comes, 1910, Boll. Accad. Gioenia Sci. Nat. Catania, (5) vol. 3, fasc. 17, p. 2; Koidzumi, 1921, Parasitology, 13:278; Kirby, 1937, Univ. Calif. Publ. Zoöl., 41:205.

Dinenymphidae Grassi and Foà, 1911, Rend. R. Accad. Lincei, (5) vol. 20, 1 sem.: 730; Poche, 1913, Arch. Protistenk., 30:152; Grassi, 1917, Mem. R. Accad. Lincei (5) 12:334; Kirby, 1931, Univ. Calif. Publ. Zoöl., 36:210; Cleveland, 1934, Mem. Amer. Acad. Arts Sci., 17:303.

*Diagnosis.*—"Polymastigote flagellates with four flagella for each nucleus (although there are occasionally eight or twelve flagella); central spindle and chromosomal fibers are intranuclear; achromatic figure and extranuclear organelles arise from intranuclear bodies which are either centrioles surrounded by centrosomes or are only centrioles . . ." (Cleveland, 1934); one axostyle, which stains heavily with Heidenhain's and delicately or not at all with Delafield's haematoxylin, is present for each daughter nucleus during the early anaphase period but this condition does not always persist through interkinesis; parabasal bodies absent; family contains three subfamilies, the Pyrsonymphinae (Kirby, 1937), the Saccinobaculinae, and the Oxymonadinae (Cleveland, 1934).

In 1877 Leidy reported two new genera, *Pyrsonympha* and *Dinenympha*, from *Reticulitermes flavipes*. Grassi (1892) placed *Pyrsonympha* Leidy pro parte in synonymy with *Dinenympha* Leidy and defined a new family, the Pyrsonymphidae, to include what he considered to be *Pyrsonympha* (Grassi nec Leidy) and *Holomastigotes* Grassi. These flagellates in which the body was "tutto coperto di flagelli" were quite unlike the original *Pyrsonympha* Leidy unless a superficial resemblance is admitted to those *Pyrsonympha* which Leidy described as "invested with cils" (pl. 52, figs. 5–7, Leidy, 1881). Even then, Grassi's choice of a name for the family seems entirely without justification unless it is assumed in addition that he intended to include as the type genus that portion of *Pyrsonympha vertens* Leidy which he had excluded from synonymy with *Dinenympha* Leidy and which was never recorded elsewhere by him. In 1911, Grassi and Foà gave the name *Spirotrichonympha flagellata* to the protozoan which Grassi had mistakenly identified as *Pyrsonympha flagellata* in 1892. They placed it and *Holomastigotes* in the family Lophomonadidae, which removed all of the explicitly named genera from the family Pyrsonymphidae and left in it only the implied type genus, *Pyrsonympha vertens* Leidy, pro parte.

When they defined the family Dinenymphidae in 1911 as containing one genus, *Dinenympha* Leidy, 1877, with which *Pyrsonympha* Leidy, 1877, and *Lophophora* Comes, 1910, were in synonymy, Grassi and Foà neglected to transfer that part of *Pyrsonympha* Leidy which they had previously assigned by implication to the Pyrsonymphidae. Possibly it was again overlooked because of its earlier, incomplete recording. A second genus, *Pseudotrypanosoma,* was added to the Dinenymphidae by Grassi in 1917, but it was removed to the Trichomonadidae by Kirby in 1931.

Poche (1913) accepted the synonymy suggested by Grassi and Foà (1911) for *Pyrsonympha* with *Dinenympha* but objected to the inclusion of *Lophophora* Comes. Koidzumi (1921) agreed to the synonymy of *Lophophora* Comes, but considered that *Dinenympha* and *Pyrsonympha* were subgenera and that *Pyrsonympha* ought to be the generic name because "the characters of the group are more typically and distinctly represented in *Pyrsonympha* than in *Dinenympha,* and the former name stands prior to the latter in the original description of Leidy (1877)." He defined the family Pyrsonymphidae Grassi as containing one genus, *Pyrsonympha,* and limited it to the characters of that flagellate. His classification is supported in part by Porter's (1897) assumption that *Dinenympha* might be the immature form of *Pyrsonympha.*

Kirby (1928) proposed the family Oxymonadidae to contain the genera *Oxymonas, Microrhopalodina,* and *Proboscidiella,* and commented on the family's close relationship to the Dinenymphidae. Cleveland (1934) retained *Pyrsonympha* and *Dinenympha* as separate genera and emended the description of the Dinenymphidae by Grassi and Foà (1911) to include the subfamilies, Dinenymphinae, Saccinobaculinae, and Oxymonadinae. He proposed this grouping largely because the production of an intranuclear central spindle, in all of them, results in a marked similarity in mitotic phenomena; and the axostyle functions in all of them as an active locomotor organelle, and consists structurally of closely appressed, paralleling fibrils. Kirby (1928) reported the similarity in structure of the axostyles of *Pyrsonympha, Oxymonas,* and *Microrhopalodina,* and their extraordinary behavior in staining heavily with Heidenhain's and slightly or not at all with Delafield's haematoxylin. Cleveland (1934) described exactly the same staining reaction for the axostyle of the Saccinobaculinae, and observation has shown that the same peculiar characteristic is present in *Barroella,* the most recently reported genus of the oxymonads. Cleveland's (1934) definition of the family was sufficiently inclusive and adequate so that only slight changes were made necessary by the results of this study of the Oxymonadinae; but he used the terms Dinenymphidae and Dinenymphinae which Koidzumi's work (1921) had discredited.

Kirby (1937) accepted Cleveland's division but corrected the family name to Pyrsonymphidae and the concomitant subfamily to Pyrsonymphinae in a table where definitions and explanations were not feasible, but his reasons were essentially as follows. Subsequent studies have maintained the genus *Pyrsonympha* undivided as it was originally presented by Leidy (1877) and have not substantiated Grassi and Foà's (1892) disposition of one part of

*Pyrsonympha* Leidy in synonymy with *Dinenympha* where it was classified in the family Dinenymphidae (1911), and of another part which was classified by implication in the family Pyrsonymphidae (1892). It is obviously impossible that *Pyrsonympha* Leidy should exist in two families. A choice between the two groups was thus obligatory, and Pyrsonymphidae was preferred because its chronological priority could not be questioned and because the absence of any other genera in it avoids additional confusion.

### Subfamily Oxymonadinae Kirby emend. Cleveland

Oxymonadidae Kirby, 1928, Quart. J. Micr. Sci., 72:356; Dubosceq and Grassé, 1934, Arch. Zool. exp. gén. 75:635. De Mello and De Mello, 1944, Anais de Instituto de Medicina Tropical, 1:227.

Oxymonadinae Cleveland, 1934, Mem. Amer. Acad. Arts Sci., 17:304; Kirby, 1937, Univ. Calif. Publ. Zoöl., 41:205.

*Type genus.*—*Oxymonas* Janicki.

*Diagnosis.*—Uni- or multinucleate Pyrsonymphidae, occurring in both motile and sessile forms in the intestine of termites of the family Kalotermitidae; xylophagous, presence or absence of wood particles within the body seemingly fortuitous; bodies usually crowded with spherules ranging in color from yellow to copper; nucleus containing a karyosome, halo, and chromatin in a granular reticulum or in granules massed against the nuclear membrane; karyosome present only in immediately postmitotic nuclei in *Oxymonas grandis;* distinguished from the Saccinobaculinae and the Pyrsonymphinae by nuclear migration during mitosis; by the presence of two groups of two flagella each, extending freely from two blepharoplasts more or less closely associated with the axostyle shoulder; by an attachment organelle, the rostellum, situated anteriorly and appearing as either a low mound or a more or less slender and lengthy, tubular or flattened anterior outgrowth of the body, at the apex of which is formed the holdfast that anchors the animal to the chitinous intima of the host's intestine; and by the presence of a less actively motile, fibrous, endoplasmic axostyle, the fibers of which are extended to form anterior and recurvent portions of varying lengths; the shape of the major portion of the axostyle resembling a slender ribbon, a stiletto, a pennant, or a scimitar, sometimes with the posterior portion protruding beyond the posterior cytoplasm; distinguished in addition to these characters from the Prysonymphinae by the absence of a spirally twisted body; and from the Saccinobaculinae by the presence of a sessile stage; possible homology existing between the short paraxostyles of the Pyrsonymphinae and the Saccinobaculinae (Cleveland, 1934) and the often lengthy, recurvent portion of the axostyle of the Oxymonadinae, these organelles being the same in their fibrous nature and in their anterior position in the body; their origin not reported in Pyrsonymphinae and Saccinobaculinae, but originating from an extension of the axostyle fibers in the Oxymonadinae (Cleveland, 1935): the subfamily consisting of three genera, *Oxymonas, Microrhopalodina,* and *Barroella* (=*Kirbyella*) (Cleveland, 1934).

Grassi and Foà (1911) described the multinucleate oxymonad, *Microrhopalodina enflata* from *Kalotermes flavicollis,* and assigned it to the Calonymphidae. Janicki (1915) reported the uninucleate animal, *Oxymonas granulosa,* from *Neotermes connexus,* and commented on its evident morphological and physiological relationship to *Microrhopalodina,* but he was reluctant to place *Oxymonas* with the latter genus until more complete studies had been made. Kofoid and Swezy (1926) described a new multinucleate genus, *Proboscidiella,* from *Cryptotermes nocens* and emphasized the evolutionary significance of its relationship to *Oxymonas.*

Bernstein (1928) listed *Oxymonas, Microrhopalodina,* and *Proboscidiella*

among the Calonymphidae but recognized that the animal figured and described by Grassi (1917) as a flask-shaped form of the calonymphid, *Stephanonympha silvestrii* from *Cryptotermes havilandi* and *Neotermes erythraeus*, was actually an *Oxymonas*. This criticism was supported by Kirby (1928), who protested additionally that the uninucleated and binucleated animal reported in the same paper to be a form of *Diplonympha foae* (a calonymphid) from *Glyptotermes parvulus* was also *Oxymonas*. Grassi (1917) maintained that these flask forms were immature specimens of the Calonymphidae; but Kirby (1928) pointed out that although similar uninucleate, flask-shaped animals were present in *Kalotermes minor* and in *K. hubbardi*, there were no *Calonymphidae* nor any other multinucleate flagellates. Kirby (1928) objected to Bernstein's taxonomic distribution and proposed the family Oxymonadidae because the relationship of *Oxymonas*, *Proboscidiella*, and *Microrhopalodina* was evident (Janicki, 1915; Kofoid and Swezy, 1926*b*; Kirby, 1928), but that of *Oxymonas* to the Calonymphidae became increasingly questionable when the "flask forms" were invalidated (Bernstein, 1928; Kirby, 1928).

Duboscq and Grassé (1934) accepted Kirby's classification, agreeing that *Oxymonas*, *Microrhopalodina*, and *Proboscidiella* belonged together and that the association with the Calonymphidae was inadvisable because the oxymonads lacked a parabasal body and had a rostellum and a dimorphic existence as motile and sessile forms. Kirby (1928) had suggested the possible synonymy of *Proboscidiella* and *Microrhopalodina*. Duboscq and Grassé objected to this, but suggested instead that *Kirbyella* Zeliff (1930) might be synonymous with *Proboscidiella*. Cleveland placed the latter genus in synonymy with *Microrhopalodina* and considered the position of *Kirbyella* (=*Barroella*) distinct but doubtful because it had been inadequately described. Kirby (1937) retained *Proboscidiella*. Cleveland's decision on *Proboscidiella* has been accepted because *Microrhopalodina* is not sufficiently different to justify their separation. The axostyle of the latter is as long as the body instead of being shorter as it is in *Proboscidiella;* and not all of the nuclei are closely associated with the shoulders of the axostyles, a condition which produces a less regular corona of nuclei than in the last-named animal. De Mello and De Mello (1944) agreed to the synonymy but violated it simultaneously and retained *Proboscidiella*.

In immature stages, *Barroella* duplicates the appearance of *Microrhopalodina;* but in the mature animal the nuclei do not form a corona but are scattered throughout the body. In *Microrhopalodina* the maximum length of the recurvent portion of the axostyle equals the length of the body but it is often made up of short, slender fibrils which are not easily distinguished. In *Barroella* the recurvent portion is a conspicuous, deep-staining, fibrous cable which greatly exceeds the length of the animal and is much looped and contorted because it lies wholly inside the body. From 1 to a little more than 50 nuclei have been reported as occurring in one animal of *Microrhopalodina*. In *Barroella*, from 2 to 114 nuclei have been recorded.

*Microrhopalodina* (Grassi and Foà, 1911) was described before *Oxymonas* (Janicki, 1915), but presumably Kirby (1928) and Cleveland (1934) have

preferred that the latter genus should furnish the stem name for the subfamily because it is a more logical choice. *Oxymonas* is better known because it occurs more frequently. Its morphology is more representative of the basic construction of the oxymonads, and the relationship of the multinucleate genus *Microrhopalodina* to the uninucleate genera of the Pyrsonymphinae and Saccinobaculinae can be realized only by tracing it through the uninucleate genus, *Oxymonas*.

## Oxymonas Janicki

*Oxymonas* Janicki, 1915, Z. wiss. Zool., 112:608 (type species, *O. granulosa*); Kofoid and Swezy, 1926, Univ. Calif. Publ. Zoöl., 28:295; Kirby, 1926, Univ. Calif. Publ. Zoöl., 29:116; 1928, Quart. J. Micr. Sci. 71:358; Bernstein, 1928, Arch. Protistenk., 61:28, 30; Zeliff, 1930, Amer. J. Hyg., 11:714; Connell, 1930, Univ. Calif. Publ. Zoöl., 36:51; Cleveland, 1934, Mem. Amer. Acad. Arts Sci., 17:303; 1935, Biol. Bull., 69:54; Cross, 1941, Univ. Calif. Publ. Zoöl., 43:379; De Mello and De Mello, 1944, Anais de Instituto de Medicina Tropical, 1:217; Nurse, 1945, Trans. Roy. Soc. New Zealand, 74:305.

*Diagnosis.*—Body form constant and symmetrical in the smaller but irregular in the larger species; spherical to elongate ellipsoidal, top-, pear-, and spindle-shaped forms; length 5–240$\mu$ in the reported species; range of average lengths 8–120$\mu$; width 4–165$\mu$; range of average widths 5–100$\mu$; predominantly uninucleate animals, binucleate specimens frequent in some species, 4, 5, and 6 nuclei occurring rarely; nucleus spherical to ellipsoidal or pyriform, long axis paralleling the body length; granular chromatin and a karyosome surrounded by a halo; cablelike portion of the spindle staining heavily with Heidenhain's haematoxylin and faintly or not at all with Delafield's stain; the number of axostyles usually being the same as the number of nuclei; nuclear matrix joining the nucleus to the shoulder of the axostyle (except in *O. notabilis*); the shape of the axostyle resembling either a slender stiletto, a pennant, or a broad scimitar, with a delicately staining anterior prolongation terminating in the holdfast or its anlage; many species (possibly all) with a recurvent portion of the axostyle extending backward from the holdfast into the body; considerable diversity in the length of the protrusion of the posterior tip of the axostyle beyond the cytoplasm, this diversity often complicated by variation depending upon the age of the animal; posterior tip of axostyle arrowhead-shaped in young animals; axostyle usually encircled by siderophile ring where it emerges from the posterior cytoplasm; pellicular symbionts either absent or significant for the differentiation of species.

Janicki's (1915) account of *Oxymonas granulosa* was followed by Kofoid and Swezy's (1926*a*) report of three new species, *O. projector, O. pediculosa,* and *O. gracilis*, and an emended definition of the genus: "Polymastigote flagellates with neuromotor system consisting of two centrosomes on the nuclear membrane connected by independent rhizoplasts with two lateral blepharoplasts from each of which three flagella arise, connected by a transverse rhizoplast in the form of a semicircle; nucleus with excentric karyosome in clear halo with a lateral granule in the halo; central axostyle continued anteriorly to apex and capable of being extended in an anterior extensile and retractile proboscis."

In 1926, Kirby described *Oxymonas parvula,* and in 1928 he suggested that "rostellum" was a more descriptive term than "proboscis" for the tubular anterior extension of the body of *Oxymonas,* and reported observations made with darkfield illumination on the flagella of living animals of four species of uninucleate oxymonads. His findings differed from those of Kofoid and Swezy (1926*a*), for in every instance he found only two flagella originating from

each of the two laterally placed blepharoplasts. Zeliff (1930*a*) described eleven new species, *O. panamae, O. kirbyi, O. clevelandi, O. brevis, O. snyderi, O. barbouri, O. joutcli, O. janicki, O. ovata, O. minor,* and *O. hubbardi,* and outlined a general scheme of mitosis for the group. He and Connell (1930) accepted the term "rostellum" that had been suggested by Kirby (1928) and reported that a barlike centrodesmose originated from the karyosome. In describing *O. dimorpha,* Connell (1930) outlined a life cycle that consisted of a flagellated motile stage and an aflagellate, sessile period. Cleveland (1934) protested that the intranuclear spindle did not originate from the karyosome and considered that Connell's error resulted from observations on overstained specimens. In 1935, Cleveland prefaced his report of *O. grandis* with a brief summary of all of the previously described species of *Oxymonas,* and emphasized the fact that in *O. grandis* the central spindle could not possibly originate from the karyosome because the latter organelle is lacking. In describing the morphology and mitosis of *O. minor* (1941), I confirmed Cleveland's (1934) contention that the intranuclear spindle did not originate from the karyosome, and postulated a post-telophase anterior migration of the nucleus preceding cytoplasmic division.

All other descriptions (Kirby, 1928; Zeliff, 1930*a*; Connell, 1930; Cleveland, 1935; Cross, 1941) except that of Kofoid and Swezy (1926*a*) and Nurse (1945) have given two flagella originating from each of the two blepharoplasts placed laterally at the base of the rostellum, and even Janicki (1915), instead of describing *O. granulosa* as "devoid of all flagella" as Kofoid and Swezy stated, said, "Immerhin habe ich Formen mit zwei Geisseln, die in zwei Basalkorperchen wurzeln, beobachtet..." He was reluctant, however, to commit himself entirely because neighboring debris on the slides offered so great an opportunity for error. This possibly explains his failure to show flagella in his illustrations, and accounts for the uncertainty whether he meant that two equaled the total number of flagella or that it was the number originating from each of the two granules. I found only one specimen of *O. granulosa* that was satisfactory for the study of flagella but in it two flagella originated from each of two very large basal granules, and from each of these blepharoplasts there also extended a sturdy rhizoplast toward the nuclear membrane.

De Mello and De Mello (1944) failed to appreciate that Kirby's (1928) generalized description of the blepharoplast-flagella complex for the subfamily, Oxymonadinae, corrected his earlier, dubiously made report of three flagella for *O. parvula.* The report by Nurse (1945) of an oxymonad in which there were two flagella from only one blepharoplast and in which each flagella was associated with an undulating membrane is erroneous (see below, p. 113).

By darkfield illumination of living animals, the flagella of *Oxymonas* are readily observable. In the attached stage the flagella are lost or diminished, and it is even difficult to find satisfactory specimens of the motile form in fixed material. Perhaps the delicacy of structure in the flagella reflects their transitory existence, for there is frequent evidence of mutilation. If a fibril passes across the body surface, it is impossible at times to determine certainly whether it is a flagellum or a fiber from the recurvent portion of the axostyle

passing backward in the cytoplasm just below the pellicle. As a consequence of these difficulties, Connell (1930) and Cleveland (1934) have questioned the advisability of stressing the flagellar complex in speciation problems.

Because of the preponderant evidence in favor of there being two flagella from each blepharoplast in *Oxymonas,* the definition has been emended accordingly. The "... two centrosomes on the nuclear membrane ...," and the "... excentric karyosome ... with a lateral granule in the halo ..." (Kofoid and Swezy, 1926*a*) have been omitted because subsequent descriptions of species have shown that these characters are not constant.

Since both the table and the review of *Oxymonas* by De Mello and De Mello (1944) are heterogenous compilations from Kofoid and Swezy (1926*a*), Kirby (1926, 1928), and Zeliff (1930*a*), additional comment would be repetitious.

Both Cleveland (1935) and Kirby (1937) questioned the validity of all eleven species of *Oxymonas* which were differentiated by Zeliff (1930*a*) largely on the basis of their occurrence in different species of termites. I have had access to the slides in Professor Kirby's collection with which Zeliff worked, and although Zeliff persistently used for diagnosis characteristics that are highly variable among the Oxymonadinae, I have found it possible to retain most of his species.

In two instances Zeliff listed the shape of the body as "distinctive" and in the small forms with which he was working this might prove feasible because they are less prone to variation in this respect than the larger oxymonads. In four species the nuclei were reported as "characteristic," a description that requires additional explanation if it is to be of value for later studies. He quite overlooked the fact that when an organelle for attachment is torn from its anchorage its "tip" may be damaged and consequently made unfit to serve as a diagnostic character. Although Kofoid and Swezy (1926) had described the rostellum as an organelle of extreme mobility that could be thrust out and retracted voluntarily, Zeliff used the length of the rostellum as a means of differentiating species. My observations on living animals do not support Kofoid and Swezy's idea of mobility, but the extreme variability in the length of the rostellum within a species is certainly made apparent by comparison of the following sketches: plate 3, figures 2, 6, 8; plate 7, figure 26; plate 9, figures 43, 44; plate 11, figures 59, 62, 64; plate 12, figures 74, 76, 77, 78, 79; plate 13, figures 83, 87; plate 14, figures 91, 92; plate 15, figures 103, 109.

In five instances Zeliff cited "distinctive size." The extreme variability in size of which these animals are capable, is demonstrated by the giants and dwarfs in plate 7, figures 26 and 30; plate 8, figures 37 and 38; and plate 15, figures 103 and 108. Examination of Zeliff's table of measurements will show how easily overlapping of species could occur with a very slight expansion of his averages. He rarely stated what the range in size was, and this would have been useful in showing how much "overlap" existed. However, in most instances, random remeasurements have established the acceptability of his averages.

Because small *Oxymonas* are usually sparsely distributed in smears, their measurement is a time-consuming matter. This, together with the fact that the expected variability in size makes it an unsatisfactory criterion for speciation, persuaded me that random measures of ten specimens would serve my purpose. I have undertaken new measurements of Zeliff's species only when my random measures have been markedly different.

Reference should be made to page 68 for an explanation of the importance of the ratios K/N and H/K in the determination of species; and to pages 92–93 for the outline of the peculiar pattern of mitosis found in the Oxymonadinae because it is essential that only interkinetic nuclei be used in a speciation problem. For example, unless the immaturity of the late reorganization stage of *Oxymonas grandis* is recognized, it might be mistaken for a separate species because of the presence of a karyosome. Nuclei that are situated posteriorly in the body cannot be considered typical; but the converse does not hold, for both prophase nuclei (pl. 9, fig. 44) and late reorganization nuclei (pl. 7, fig. 23; pl. 13, fig. 88) may occur in the customary interkinetic position of the nucleus at the base of the rostellum.

KEY TO THE SPECIES OF OXYMONAS

1. Axostyle broad, resembling a scimitar in shape ..... 2
   Axostyle slender, resembling a stiletto in form ..... 3
   Axostyle pennant-shaped, with shoulder retracted into body and recurvent portion deeply stained ..... *O. notabilis*
2. Karyosome absent in nucleus during interkinetic period, range in length 41–241$\mu$ *O. grandis*
   Karyosome usually ovoid and large in proportion to the size of the nucleus, length 25–200$\mu$ ..... *O. megakaryosoma*
3. Position of karyosome central or slightly excentric, chromatin granules massed against the nuclear membrane ..... 4
   Position of karyosome central or slightly excentric, chromatin in granular reticulum 6
   Position of karyosome definitely excentric, chromatin in granular reticulum ..... 8
   Karyosome minute, chromatin in fine granules distributed throughout the nucleus, body ovoid, range in length 5–13$\mu$; halo usually absent ..... *O. parvula*
4. Karyosome oval, body ovoid, axostyle projecting slightly beyond the posterior cytoplasm, K/N = 1/2, H/K = 1/2 ..... *O. ovata*
   Karyosome round, infrequently oval, body spindle to top-shaped ..... 5
5. Karyosome usually round, axostyle projecting moderately beyond posterior cytoplasm, K/N = 1/5, H/K = 1/–1 ..... *O. brevis*
6. Body elongate, slender top-shaped, axostyle projecting moderately beyond posterior cytoplasm, K/N = 1/3, H/K = 1/1 to 1/2 ..... *O. jouteli*
   Body subspheroidal to ellipsoidal with pointed posterior, axostyle projecting slightly beyond the posterior cytoplasm, K/N = 1/4, H/K = 1/1, matrix sleeve production frequent ..... *O. projector*
   Body broadly ovoidal, axostyle projecting slightly beyond posterior cytoplasm .... 7
7. Karyosome oval, length of body 7–28$\mu$; K/N = 2/5, H/K = 1/2 ..... *O. barbouri*
   Karyosome round, length of body 27–40$\mu$, K/N = 1/3, H/K = 1/3 to 2/3 ... *O. pediculosa*
8. Body top- or spindle-shaped with pointed posterior ends ..... 9
   Body spheroidal to ellipsoidal, occasional spindle forms, but posterior end not pointed ..... 11
9. Body top-shaped, heads of axostyles "beaded" ..... 10
   Body plump spindle-shaped, width ⅔ of length, axostyle projects slightly beyond posterior cytoplasm, K/N = 1/4, H/K = 1/1 to 5/2 ..... *O. rotunda*

Body spindle-shaped, axostyle projecting much beyond the posterior cytoplasm, K/N = 1/3, H/K = 1/2 to 2/3 ..... *O. caudata*

10. Axostyle projecting slightly beyond posterior cytoplasm, K/N = 1/3, H/K = 4/5 to 2/1 because of halo's asymmetry, pellicular symbionts consisting of short, plump rods ..... *O. clevelandi*

More slender, axostyle projecting moderately beyond the posterior cytoplasm and broadened from its midpoint anteriorly, K/N = 1/3, H/K = 1/2, pellicular symbionts consisting of long spirochaetes and long, curved rods ..... *O. hubbardi*

11. Body ovoidal, expanded posteriorly, length 7–16$\mu$, axostyle projecting moderately beyond posterior cytoplasm, K/N = 1/4, H/K = 1/1 ..... *O. snyderi*

Body ovoidal, length 8–25$\mu$, axostyle projecting very slightly beyond posterior cytoplasm, K/N = 1/5, H/K = 1/1 ..... *O. gracilis*

Body ovoidal, length 13–48$\mu$, axostyle projecting slightly beyond posterior cytoplasm, K/N = 2/5, H/K = 1/3, matrix sleeves frequent ..... *O. kirbyi*

Body ovoidal, sometimes pyriform, length 8–40$\mu$, axostyles frequently shorter than body, K/N = 1/3, H/K = 1/1, pellicular symbionts consisting of cocci found infrequently ..... *O. di-undulata*

Body ovoidal, sometimes spindle-shaped, length 5–30$\mu$, K/N = 1/3 to 1/4, H/K = 1/1 to 2/3 ..... 12

12. Body spheroidal to ovoidal, nucleus broadly ovoidal, membrane heavy, axostyle with a sharp curve across the nucleus, blepharoplasts extraordinarily large ... *O. granulosa*

Body spherical to ellipsoidal, sometimes spindle forms but with posterior not pointed, nucleus spherical to pyriform, matrix sleeves not infrequent ..... *O. minor*

## Oxymonas granulosa Janicki

*Oxymonas granulosa* Janicki, 1915, Z. wiss. Zool., 112:608, pls. 13–18.

*Type host.*—*Neotermes connexus* Snyder. Hawaii. (Incorrectly named *N. castaneus* Burmeister by Janicki.)

T-305. (Xenosyntype slide TP-199:13.)

T-306. (Xenosyntype slide TP-208:4.)

*Diagnosis.*—Body spherical to ovoid; (T-305) length 7.9(5.3–13.3)$\mu$; width 4.7(4.0–8.8)$\mu$; (T-306) length 21.0(17.2–30.0)$\mu$; width 12.2(9.1–13.6)$\mu$ (Cross); length 23$\mu$ (Janicki); nucleus broadly ovoid, averaging (T-305) 2.9 × 2.5$\mu$; (T-306) 4.3 × 4.0$\mu$ (Cross); well-defined membrane, finely granular reticulum of chromatin; karyosome round, surrounded by halo, excentric posteriorly; K/N = 1/3; H/K = 1/–1; blepharoplasts extraordinarily large; axostyle slender, with a pronounced curvature around the nucleus just below the shoulder, projecting posteriorly a moderate distance beyond the cytoplasm; no pellicular symbionts.

I measured 10 specimens from each of two slides. Slide TP-199:13 contained much smaller animals than Janicki reported, but slide TP-208:4 furnished specimens the average length of which approximated that reported by him if it is presumed that his measurement did not include the rostellum. These results do not support his statement that *Oxymonas* "variiert wenig in der Grosse" but show that *O. granulosa* also conforms to the wide range in size found in subsequently described species. The specific name was chosen because Janicki considered that the cytoplasmic spherules were the most striking characteristic of the species. He believed that the "Kugeln" were metabolic products derived from the wood particles which were ingested through the surface of the body. His description of the axostyle, blepharoplasts, and nucleus is fairly satisfactory, and his figures of them are acceptable as diagrammatic representations. His discussion of the flagella was not adequate, as he himself complained, but his description of the attachment of

*Oxymonas* to the gut lining of the host "mit Hilfe ihres spitz ausgezogenen Vorderendes" tells as much as can be learned from observation of the smaller oxymonads.

In the nucleus Janicki stressed the presence in the halo of a spherical or diploid body which he called the "entosome" and which he suspected of producing the spindle. He concluded, however, that the karyosome was a more logical source of the spindle because its staining reactions were more similar. His entosomes were probably somewhat isolated granules of chromatin which have been reported (Zeliff, 1930; Cross, 1941) as occurring during the prophase in double strands. More recent work did not confirm his supposition that the karyosome might be the source of the spindle (Cleveland, 1934, 1935; Cross, 1941); nor that the old spindle became the axostyles (Zeliff, 1930; Connell, 1930; Cleveland, 1935; Cross, 1941). Undoubtedly the granules that he called extranuclear centrioles are the axostyle anlagen, and there is no longer any question but that spindle formation is intranuclear. His query in respect to the homology of the axostyle of *Oxymonas* with that of *Dinenympha* Leidy was answered in the affirmative by Kirby (1928) and Cleveland (1934).

Janicki's description and figures of a "transparenten Plasmaportion" which invariably capped the poles of the dividing nucleus during telophase and persisted for some time thereafter in a less readily recognizable state, represent, evidently, one aspect of the cyclically polymorphous component which was called the "clear area" by Connell (1930), a "halo surrounding the nucleus" by Cleveland (1934), and which I now term "nuclear matrix." Janicki did not detect the presence of nuclear migration during mitosis, but even though his mitotic series was incomplete, he recognized that the pattern of reproduction in *Oxymonas* was sharply dissimilar from that of other flagellates.

### **Oxymonas projector** Kofoid and Swezy

*Oxymonas projector* Kofoid and Swezy, 1926, Univ. Calif. Publ. Zoöl., 28:287, pl. 30, figs. A, B, C.

*Type host.*—*Kalotermes perparvus* Emerson. Kartabo, British Guiana.

*Diagnosis.*—Body subspheroidal to subellipsoidal and pointed posteriorly; length 12–40$\mu$; nucleus spherical, chromatin in coarsely granular reticulum; karyosome round, surrounded by halo, more or less centrally situated; K/N = 1/4; H/K = 1/1; two lateral blepharoplasts at shoulder of axostyle, joined by a semicircular fibril, each with a rhizoplast extending to the nuclear membrane; axostyle slender, projecting slightly beyond the posterior cytoplasm; matrix sleeve not uncommon; no cytoplasmic spherules; no pellicular symbionts reported.

The diagnosis is based on Kofoid and Swezy's description, but they overemphasized the importance of the neuromotor system, believing it manipulated the extension and retraction of the "proboscis." The "retractor fibers" reported by them belong undoubtedly to the recurvent portion of the axostyle, and morphologically their axostyle sleeve is comparable to the "sleeve" produced by the nuclear matrix. Observations on other *Oxymonas* have not supported their statement that the sleeve vanishes when the rostellum is extended. In general, immature forms have no rostella and the matrix sleeve appears more commonly in young animals. There were no studies of mitosis,

but plate 30, figure 5 (Kofoid and Swezy, 1926) might very easily be an illustration of the nucleus of a very young oxymonad rather than an optical cross section of the sleeve. A discussion of their report that three flagella originate from each of the two blepharoplasts has been given on page 98.

### **Oxymonas pediculosa** Kofoid and Swezy

*Oxymonas pediculosa* Kofoid and Swezy, 1926, Univ. Calif. Publ. Zoöl., 28:296, pl. 30, fig. 11.

*Oxymonas panamae* Zeliff, 1930, partim, Amer. J. Hyg. 11:726, pls. 1, 2, 4.

*Type host.*—*Kalotermes nigriceps* Emerson. Kartabo, British Guiana.

*Additional host.*—*Rugitermes panamae* Snyder. Panama.

T-191. Barro Colorado. (Homosyntype slide TP-111:20.)

*Diagnosis.*—Body broadly ovoidal; length 34(27–40)$\mu$; width 21(15–25)$\mu$ (Kofoid and Swezy); nucleus spherical, diameter 7$\mu$ (Cross), chromatin in granular reticulum; karyosome round, surrounded by a halo, slightly excentric; K/N = 1/3; H/K = 1/3 to 2/3; axostyle slender, projecting very slightly beyond the posterior cytoplasm; body covered densely with spirochaetes about 5$\mu$ long (Cross), with shorter, broad, curved rods attached by one end, and with small flagellates interspersed occasionally among these.

The diagnosis is based on Kofoid and Swezy's description. My comments on their overemphasis of the neuromotor system in *Oxymonas projector* (above, p. 102) are equally applicable to this species, and reference is again made to page 98 for a discussion of their report that three flagella originate from each blepharoplast.

Zeliff commented on the similarity of *O. panamae* to *O. pediculosa* but he stressed the difference which he found in their size. Zeliff's average for *O. panamae* was 18 × 26$\mu$. Because my random measurements are in agreement with those of Kofoid and Swezy, and because their description conformed to my observations in all other respects excepting the number of flagella, there seems insufficient reason for maintaining two species. Moreover, there is the possibility of inaccuracy in the flagellar number as reported by Kofoid and Swezy, for they stated that the dense covering of bacteria made all morphological studies difficult and that specimens occurred infrequently in the smears. My experience with the species from *R. panamae* paralleled theirs. Zeliff, however, found flagella that ranged in length from 50 to 70$\mu$. Kofoid and Swezy made no studies of mitosis. Zeliff illustrated numerous mitotic stages and stated that there "appear to be about seventeen chromosomes." Presumably much of his summary of mitosis in the genus is based on this species.

### **Oxymonas gracilis** Kofoid and Swezy

*Oxymonas gracilis* Kofoid and Swezy, 1926, Univ. Calif. Publ. Zoöl., 28:297, pl. 30, figs. 9–10.

*Type host.*—*Kalotermes magninotus* Emerson. Kartabo, British Guiana.

*Diagnosis.*—Body ovoidal to ellipsoidal; length 15(8–25)$\mu$; width 8.5(4–15)$\mu$ (Kofoid and Swezy); nucleus spherical to ovoidal; chromatin in granular reticulum; karyosome round, surrounded by halo, excentric posteriorly; K/N = 1/5; H/K = 1/1; axostyle slender and projecting very slightly beyond the posterior cytoplasm; no pellicular symbionts reported.

The diagnosis is based on Kofoid and Swezy's description. My discussion of their overemphasis of the neuromotor system in *Oxymonas projector*

(above, p. 102) is also applicable to this species, and reference is made to page 98 for comments on their report that three flagella originate from each blepharoplast. In Kofoid and Swezy's six illustrations of *O. gracilis*, the presence of an arrowhead-shaped tip on the only axostyle that protruded noticeably beyond the posterior cytoplasm implied that the animal was immature and consequently disqualified to serve as a type specimen. Only three slides were available for the study of this species, but *Oxymonas gracilis* was "relatively more abundant" than either *O. projector* or *O. pediculosa*.

### Oxymonas parvula Kirby

*Oxymonas parvula* Kirby, 1926, Univ. Calif. Publ. Zoöl., 29:116, pl. 9, figs. 21–23.

*Type host.*—*Cryptotermes hermsi* Kirby. Fanning Island.

T-9. (Xenosyntype slide TP-1:1.)

*Diagnosis.*—Body slender, ovoid, posteriorly truncate, rounded or pointed; length 5–13$\mu$ (Kirby); nucleus round, chromatin finely granular and distributed throughout the nucleus; karyosome minute, halo much diminished or lacking; K/N = 1/8; axostyle slender, projecting slightly beyond the posterior cytoplasm; pellicular symbionts consisting of a posterior fringe of spirochaetes.

The diagnosis is based on Kirby's sketches and descriptions, and is confirmed by my own observations. By inference, Kirby (1928) corrected his earlier, dubiously reported account of the presence of three flagella with each of the two blepharoplasts when he stated, "In features other than the flagella, the uninucleate oxymonads which the writer has observed resemble those described by Kofoid and Swezy." *O. parvula* is smaller than any of the other species except the small forms of *O. granulosa* from which they are readily distinguished by their more slender body, by their very small karyosome, and by the absence or extreme diminution of the halo.

### Oxymonas caudata sp. nov.

*Oxymonas panamae* Zeliff, 1930, partim, Amer. J. Hyg., 11:726.

*Type host.*—*Kalotermes peresi* Holmgren. Costa Rica.

T-263. Cartago. (Syntype slide TP-222:36.)

*Diagnosis.*—Body broadly spindle-shaped, posteriorly elongate and pointed; length 17.8(10.0–20.8)$\mu$; width 11.8(7.0–14.5)$\mu$ (Cross); nucleus spherical to pyriform, approximate diameter 4.5(3.7–7.0)$\mu$ (Cross); chromatin in coarsely granular reticulum; karyosome round, surrounded by halo, excentric posteriorly; K/N = 1/2, sometimes 1/3; H/K = 1/2 to 2/3, because the halo encircles the karyosome asymmetrically; axostyle slender, broadened slightly at the shoulder and tapering sharply as it curves across the nucleus; protruding about 5$\mu$ beyond the posterior cytoplasm, but appearing longer because of the pointed posterior extension of the body; tapering sharply to a point after leaving the cytoplasm; cytoplasmic spherules very small; pellicular symbionts consisting of a dense coat of spirochaetes about 9$\mu$ in length.

Zeliff's average measurements for the species exceeded those of the largest specimens that I found; and, contrary to his report, the animals occurred abundantly. However, the separation of the *Oxymonas* found in *Kalotermes perezi* from the *Oxymonas* in *Rugitermes panamae* was not made on the basis of size alone. Among the oxymonads, the protrusion of the axostyle usually indicates immaturity, but did not do so in this instance. Instead, it is the most

distinctive characteristic of the species and occurs invariably. The broadening of the axostyle below the shoulder in *O. caudata,* the larger ratio of K/N, and the posterior position of the karyosome were additional well-defined differences. The pellicular spirochaetes are almost twice as long as in *O. pediculosa,* and the thick curved rods and small flagellates are lacking. The recurvent portion of the axostyle was identified in one specimen. In view of Zeliff's statement that this animal is scarce, it is unlikely that his report on the chromosome number of *O. panamae* referred to *O. caudata.*

### Oxymonas kirbyi Zeliff

*Oxymonas kirbyi* Zeliff, 1930, Amer. J. Hyg., 11:727.

*Oxymonas janicki* Zeliff, 1930, Amer. J. Hyg., 11:729.

*Type host.*—*Rugitermes kirbyi* Snyder. Costa Rica.
T-135. Cartago. (Xenosyntype slide TP-76:19.)

*Diagnosis.*—Body broadly ovate; length 29.3(13–48)$\mu$; width 13.2(8.3–21.6)$\mu$ (Cross); nucleus ovoid, $7 \times 5.5\mu$ (Zeliff); chromatin in granular reticulum; karyosome round, surrounded by a halo, posteriorly excentric; K/N = 2/5; H/K = 1/2; axostyle slender and projecting very slightly beyond the posterior boundary of the body; recurvent portion of the axostyle frequently found; matrix sleeves common; pellicular symbionts consisting of rods, usually attached by end, about 1.3$\mu$ long, and slender spirochaetes about 4$\mu$ long.

Zeliff (1930) described *O. janicki* as a second species solely because its measurements were smaller. Intermediates in size were readily found in my examination of the material, and a consideration of less variable characteristics established its identity with *O. kirbyi* which has page priority in Zeliff's account. The appearance of the nucleus and the tendency to produce matrix sleeves was the same in both. Although *O. kirbyi* is comparatively small, the recurvent part of the axostyle could frequently be discovered in both the large and the small forms. The latter characteristic distinguished it from all other *Oxymonas* in the group that have slender axostyles, and that feature in company with the tendency toward the production of matrix sleeves is particularly striking in animals that have been fixed with osmic vapor. With that fixative two large blepharoplasts could readily be seen lying in the position usual with *Oxymonas* at the base of the rostellum and in close association with the shoulder of the axostyle. Each consisted of two large spherical granules of which the more distal in each gave origin to two flagella. Other fixatives gave far less satisfactory definition of the blepharoplast-flagella complex.

The nuclear matrix is often readily distinguished. The nuclear membrane is well defined and the chromatin is in coarse granules evenly distributed in a fine-meshed reticulum. Zeliff reported mitosis in this species and commented upon the large size of the nucleus relative to the size of the body.

### Oxymonas clevelandi Zeliff

*Oxymonas clevelandi* Zeliff, 1930, Amer. J. Hyg., 11:228.

*Type host.*—*Kalotermes clevelandi* Snyder. Panama.
T-235. Barro Colorado. (Xenosyntype slide TP-147:2.)

*Additional host.*—*Kalotermes tabogae* Snyder. Panama.
T-227. Taboga Island. (Homosyntype slide TP-136:41.)

*Diagnosis.*—Body slender top-shaped; length 17(14–20)$\mu$; width 8(7–10)$\mu$ (Zeliff); nucleus spherical to pyriform, 3 × 4$\mu$ (Zeliff); chromatin in coarse, granular reticulum; karyosome round, surrounded by halo, excentric posteriorly; K/N = 1/3; H/K = 4/5 to 2/1 because the halo encircles the karyosome asymmetrically; axostyle slender, projecting slightly beyond the posterior cytoplasm; head of axostyle appears "beaded"; pellicular symbionts consisting of very short, plump rods.

Zeliff's measurements were computed from 75 animals from *Kalotermes clevelandi*. The average length for 25 specimens from *K. tabogae* exceeded that of specimens from *K. clevelandi* by 3$\mu$, a difference which Zeliff considered slight enough to justify the acceptance of measurements from *K. clevelandi* as typical of the species. Although he did not report them, there are reasons in addition to their similarity in size for placing the *Oxymonas* from *K. clevelandi* and from *K. tabogae* in the same species. In both, the posterior part of the nucleus appears bulbous and clear because a large volume is occupied by the halo substance. Anteriorly from the karyosome the halo is narrow, but laterally and posteriorly it is widened. Sometimes during the prophase or the reorganization following mitosis, the nuclei of other species appear to be similar, but the characteristic is coupled with degenerating or regenerating axostyles and is not persistent as it is in *O. clevelandi*. Only *O. hubbardi* also has the "beaded" axostyle head which results from the occurrence of a short chain of large cocci just below the shoulder of the axostyle and parallel to its length. This localization is quite as definitely and narrowly limited as the spirochaetes reported as symbionts of *O. grandis* (Kirby, 1941). The characteristics which differentiate *O. clevelandi* from *O. hubbardi* are discussed with the latter species (below, p. 110). The blepharoplasts were reported by Zeliff as spherical. Kirby (1928) published sketches made from darkfield illumination of living specimens from *K. tabogae* which showed that two flagella, about twice the length of the body, originate from each blepharoplast. In one instance the recurvent part of the axostyle was unmistakably present. It probably occurs in all specimens, but in small oxymonads is not readily distinguished because of its delicacy.

### Oxymonas brevis Zeliff

*Oxymonas brevis* Zeliff, 1930, Amer. J. Hyg., 11:728.

*Type host.—Cryptotermes brevis* Walker. Puerto Rico; Peru.

T-4600. Lima. (Xenosyntype slide TP-3283:3.)

*Diagnosis.*—Body sometimes spindle- but usually top-shaped; length 13(Zeliff) (6.7 [Cross]–19[Zeliff])$\mu$; width 5$\mu$ (Zeliff); nucleus round, rarely elongate, diameter 3$\mu$ (Zeliff, Cross); chromatin massed heavily against nuclear membrane; karyosome round, but occasionally oval, more or less centrally situated; diameter of karyosome 0.6$\mu$, surrounded by halo; width of halo 0.7$\mu$ (Cross); K/N = 1/5; H/K = 1/–1; axostyle slender and projecting a moderate distance beyond the posterior boundary of the body; no pellicular symbionts.

The nucleus resembles a very young nucleus of *Oxymonas minor* but the body of the latter during immaturity is broadly ovoidal or spherical and quite unlike the slender top or spindle forms of *O. brevis*. The size approaches that of *O. parvula*, but the massing of the chromatin against the nuclear membrane

and the distinct halo around the karyosome in the former species, as well as the contrasting shapes of the bodies, establish their differentiation.

Remeasurements of the karyosome and the width of the halo were made in 10 specimens because Zeliff's report of 0.25$\mu$ for the breadth of the karyosome seemed questionable. His statement that the karyosome was small must be admitted, but in relation to the size of the nucleus it is not extraordinarily so, for the ratio of K/N is 1/5. The ratios for K/N and H/K are identical with those for *O. gracilis*, from which *O. brevis* is again differentiated by the massing of the chromatin against the nuclear membrane and by its slender top-shaped body.

### Oxymonas synderi Zeliff

*Oxymonas snyderi* Zeliff, 1930, Amer. J. Hyg., 11:728.

*Type host.*—*Cryptotermes breviarticulatus* Snyder. Panama.
T-230. Taboga Island. (Xenosyntype slide TP-141:11.)

*Diagnosis.*—Body ovoidal, expanded posteriorly; length 12.5(7–16)$\mu$; width 7.9(4.7–9.3)$\mu$ (Cross); nucleus ovoid, length 4.5(3.2–7.3)$\mu$; width 3.2(2.7–4)$\mu$ (Cross); chromatin in granular reticulum; karyosome round, diameter 0.8$\mu$ (Cross), surrounded by a halo, width of halo 0.8$\mu$ (Cross), excentric posteriorly; K/N = 1/4; H/K = 1/1; axostyle slender, projecting moderately beyond the posterior boundary of the body; pellicular symbionts consisting of numerous slender spirochaetes, 3–7$\mu$ in length.

I did not find, as Zeliff did, that the size was uniform in this species and my averages were consistently greater as a consequence of my inclusion of larger animals. I did not find that the nucleus was round (Zeliff's sketches show them ovoid) except in a few specimens in which other characteristics also implied their immaturity. Zeliff commented on the similarity of *Oxymonas snyderi* to *O. brevis* and separated them into two species with the insufficient explanation that the "... karyosome is slightly more prominent than in *O. brevis* ... The rostellum is longer ... and the tip is pointed." *O. snyderi* has a pyriform body, the nucleus is ovoid, with the karyosome situated posteriorly, and the ratio of K/N = 1/4, which are all in contrast to *O. brevis* with its spindle- or top-shaped body, its round nucleus with the karyosome in a nearly central position, and its ratio of K/N equaling 1/5. The last-named species has no pellicular symbionts, but *O. snyderi* is covered with long spirochaetes. Remeasurements of the karyosome and of the width of the halo were made in 10 specimens because Zeliff's report of 0.33$\mu$ for the breadth of the karyosome seemed questionable. The blepharoplasts were described by him as spherical. Mitotic figures were reported as having been seen, but the two sketches are too small to illustrate more than the posterior position usually assumed by the dividing nucleus.

### Oxymonas barbouri Zeliff

*Oxymonas barbouri* Zeliff, 1930, Amer. J. Hyg., 11:729.

*Type host.*—*Glyptotermes barbouri* Banks. Panama.
T-127. Barro Colorado. (Xenosyntype slide TP-65:6.)

*Diagnosis.*—Body broadly ovoidal in both large and small animals; rounded posteriorly, but with a short, sharp extension where the axostyle emerges from the cytoplasm; length 16.1(6.7–27.8)$\mu$; width 11.0(4.7–14)$\mu$ (Cross); nucleus usually round, occasionally ovoid; diameter 4$\mu$ (Zeliff, Cross); chromatin in granular reticulum; karyosome usually oval,

sometimes roughly triangular, sometimes round, sometimes with the long axis parallel to the long axis of the body, sometimes with it perpendicular, surrounded by a halo, position slightly excentric; K(length)/N (width) = 2/5; H/K(length) = 1/2; axostyle slender, not tapering posteriorly, broadened slightly as it crosses the nucleus, projecting slightly beyond the posterior cytoplasm; pellicular symbionts consisting of spirochaetes about 6$\mu$ in length and distributed more densely over the posterior third of the body.

My observations confirmed Zeliff's statement of the scarcity of specimens of *O. barbouri,* but his report of the uniformity of size in the species is true only if the measurements are confined to one slide. When several slides were investigated, the results showed that within the host species the range in size was wide; and that the limited range within the individual host implied that the cyclical development of *O. barbouri* was highly synchronized. Zeliff reported that the blepharoplasts were spherical, but he failed to notice that the karyosome was oval. The latter characteristic and the generous width of the body in proportion to its length are the most outstanding features of the species. Except for one specimen, the pellicular symbionts were too pale to permit satisfactory studies; but the accumulation of evidence justifies the assumption that the pellicular symbionts portrayed with the well-stained animal is typical for the species.

### Oxymonas jouteli Zeliff

*Oxymonas jouteli* Zeliff, 1930, Amer. J. Hyg., 11:729.

*Type host.—Kalotermes jouteli* Banks. Florida; Mexico.

T-4614. Englewood. (Xenosyntype slide TP-3294:10.)

*Diagnosis.*—Body usually elongate top-shaped, tapering to a point posteriorly; length 20.9(13.3–30.7)$\mu$; width 8.5(5.3–18.0)$\mu$ (Cross); nucleus ovoid, 4 × 5$\mu$ (Cross); chromatin in coarsely granular reticulum; karyosome round, surrounded by halo, usually slightly excentric, sometimes posteriorly excentric; K/N = 1/3; H/K varies from 1/1 to 1/2; axostyle slender, projecting moderately beyond the posterior cytoplasm; no pellicular symbionts.

Contrary to Zeliff's report, a large number of specimens were present in the smears, rostella were seen in a number of animals, the size range was wide, and the average sizes proved to be smaller for the body and larger for the nucleus. In general, the rostella were about equal to the length of the body, but in one instance the length was 46.7$\mu$. The numerous small forms, unlike the rotund immature animals of other oxymonads, were relatively more slender and maintained their elongate top shape more consistently than the larger specimens did. The relations of the karyosome in the nuclear structure were difficult to evaluate unless it was presumed that the variation in size and position found in the small specimens was not typical but was indicative of their immaturity. Curiously, the usually stable ratios K/N and H/K have proved to be less so in *Oxymonas jouteli,* and the customary variability among the oxymonads in the shape of the body was absent in the smaller specimens and much diminished in the larger forms of this species.

### Oxymonas ovata Zeliff

*Oxymonas ovata* Zeliff, 1930, Amer. J. Hyg., 11:730.

*Type host.—Calcaritermes brevicollis* Banks. Panama.

T-233. Barro Colorado. (Xenosyntype slide TP-144:8.)

*Diagnosis.*—Body ovoid; length 11.9(5.3–16.6)$\mu$; width 6.7(4.0–8.7)$\mu$ (Cross); average 15.5 × 19$\mu$ (Zeliff); nucleus round; diameter 4$\mu$; chromatin granules massed closely against nuclear membrane; karyosome ovoid, centrally situated; surrounded by a halo; K/N = 1/2; H/K = 1/2; axostyle slender, projecting very slightly beyond posterior cytoplasm; pellicular symbionts consisting of a sprinkling of large diplococci.

My observations support the suitability of Zeliff's choice of *ovata* as the species name for the oxymonad from *Calcaritermes brevicollis,* but they do not sanction his placing the *Oxymonas* from *C. emarginicollis* in the same species. My specimens in three smears from *C. brevicollis* were much smaller than those described by Zeliff. The massing of the chromatin against the nuclear membrane, and the large, oval, centrally situated karyosome are characteristics associated with immaturity in most of the oxymonads; but in *O. ovata* this arrangement of the nuclear components appeared consistently. In the nuclei of the oxymonad from *C. emarginicollis,* however, the chromatin occurred in a granular reticulum and the karyosome was only about one-fourth as wide as the nucleus. No nuclei of this description were seen in either smears or sections from *C. brevicollis.* In both flagellates the body is broad in proportion to its length, but in *O. rotunda* it is widest near its midpoint and tapers both posteriorly and anteriorly to produce a plump spindle shape. In *O. ovata* the breadth is maintained as a wide girdle through the central region, beyond which the body is bluntly rounded off at the anterior and posterior ends to produce an ovoid form. Occasionally, a plump, spindle-shaped animal occurs in *O. ovata,* but the nuclei of the two species are sufficiently different to avert confusion. Unlike Zeliff's experience, specimens of *O. ovata* were found abundantly in smears. The nucleus of *O. ovata* resembles that of *O. barbouri* in having an oval karyosome, but differs in having the chromatin granules massed against the nuclear membrane rather than disposed in a granular reticulum.

### **Oxymonas rotunda** sp. nov.

*Oxymonas ovata* Zeliff, 1930, partim, Amer. J. Hyg., 11:730.

*Type host.—Calcaritermes emarginicollis* Banks. Costa Rica.
T-171. Estrella. (Syntype slide TP-106:18.)

*Diagnosis.*—Body plump spindle-shaped; length 18.5(10.7–27)$\mu$: width 12.5(6–15.5)$\mu$ (Cross); average (Zeliff) 15.5 × 19$\mu$; nucleus spherical to ovoid, 4.6 × 3.3$\mu$ (Cross); chromatin in coarsely granular reticulum; karyosome round, excentric either posteriorly or anteriorly, surrounded by a halo; K/N = 1/4; H/K = 1/1 to 5/2 because the halo encircles the karyosome asymmetrically; axostyle slender, projecting slightly beyond the posterior boundary of the body; pellicular symbionts consisting of a covering of plump rods about 0.7$\mu$ in length, sometimes attached by the end and sometimes by the side.

My observations of *O. rotunda* agree approximately with Zeliff's report of *O. ovata* in respect to the scarcity of specimens in the smears and in the average size. A plump spindle shape like that of *O. rotunda* is sometimes found in *O. barbouri,* but the oval karyosome of the latter distinguishes it. The karyosomes are round and the chromatin in the nuclei of *O. pediculosa, O. snyderi,* and *O. jouteli* is disposed similarly to that in *O. rotunda,* but in the former species the shapes of the bodies, and the ratios of K/N and H/K are very different. *O. pediculosa* has a broadly ovoidal body; *O. snyderi* has an ovoidal

form, expanded posteriorly; and *O. jouteli* has a slender, elongate top shape. The length, as indicated by the range, is much less than in *O. pediculosa,* and much greater than in *O. snyderi.* The characteristics of *O. rotunda* which distinguish it from *O. ovata* have been discussed in connection with the latter species.

### Oxymonas minor Zeliff

*Oxymonas minor* Zeliff, 1930, Amer. J. Hyg., 11:730; Cross, 1941, Univ. Calif. Publ. Zoöl., 43:379.

*Type host.—Kalotermes minor* Hagen. California.

T- Los Angeles. (Xenosyntype slide TP-560:1.)

*Diagnosis.*—Body spherical to elliptical, broad to slender spindle forms, usually rounded posteriorly; length 24.7(5.8–30.8)$\mu$, giant 81$\mu$; width 13.2(5.8–17.2)$\mu$ (Cross); nucleus spherical to pyriform, 6.3 × 4.7$\mu$ (Cross); chromatin in granular reticulum; karyosome round, surrounded by halo, excentric posteriorly; K/N = 1/4 to 1/3; H/K = 1/1 to 2/3; axostyle slender, projecting very slightly beyond posterior cytoplasm; pellicular symbionts consisting of a fringe of spirochaetes along the posterior boundary of the body, often absent or insufficiently stained.

The average size of the body and the length of the flagella agree rather closely with Zeliff's report, but according to my observations the nucleus was both broader and shorter. Although he commented on the variability in the size of the body, Zeliff published no maximums nor minimums and described the size as "characteristic." The blepharoplasts are not spherical, as he described them, but each consists of two closely appressed granular spheres. A detailed description of the morphology has been given in an earlier paper (Cross, 1941).

### Oxymonas hubbardi Zeliff

*Oxymonas hubbardi* Zeliff, 1930, Amer. J. Hyg., 11:730.

*Type host.—Kalotermes hubbardi* Banks. Arizona.

T-252. (Xenosyntype slide TP-189:15.)

*Diagnosis.*—Body plump, top-shaped, sometimes ovoid, pointed posteriorly; length 14.5 (7.3–21.4)$\mu$; width 9.4(5.5–10.7)$\mu$ (Cross); average 17 × 8$\mu$ (Zeliff); nucleus ovoid, 4.5 × 3.5$\mu$ (Cross); chromatin in granular reticulum; karyosome round, surrounded by a halo, excentric posteriorly; K/N = 1/3; H/K = 1/2; axostyle slender, the anterior half broadened, projecting posteriorly about 3.5$\mu$ (Cross) beyond the cytoplasm; head of axostyle appears "beaded"; pellicular symbionts consisting of a dense covering of long spirochaetes and equally long rods, with the greatest density at the base of the rostellum.

Both *Oxymonas hubbardi* and *O. clevelandi* have a short chain of cocci closely associated with the axostyle just below the shoulder and paralleling its length, which gives the axostyle a "beaded" appearance. Zeliff did not notice this similarity between the two species but commented on their resemblance without any supporting statement, unless the fact that his averages in size were identical can be so construed. He separated the two species because "... the rostellum is shorter and stouter and seems constant enough to be considered a differential character in this case without additional characters." *O. hubbardi* has an ovoid nucleus; *O. clevelandi,* a spherical or pyriform one. In both, the karyosome is excentric posteriorly and the ratios of K/N are alike. The posterior region of the nucleus of *O. clevelandi* appears bulbous because

of the large volume of clear halo substance. The halo encircles the karyosome asymmetrically. Neither appearance is true for *O. hubbardi.* The latter has a broad top shape, and the length is 1.7 times its width. The other has a slender top shape and the length is 2.1 times the width. The axostyle of *O. hubbardi* projects noticeably beyond the posterior cytoplasm and is broadened gradually from its midpoint anteriorly. In addition to the last-named characteristics and the absence of a "beaded" shoulder in the axostyle, the body of *O. minor* differs from that of *O. hubbardi* in being spindle-shaped rather than top-shaped. The dense growth of spirochaetes and long rods covering *O. hubbardi* is very different from the coating of plump rods on *O. clevelandi* and the localized posterior fringe of spirochaetes on *O. minor.*

Zeliff reported that the blepharoplasts were spherical. Kirby (1928) determined by means of darkfield illumination with living specimens that two flagella, which were from 2 to 2½ times the length of the body, originated from each of the two blepharoplasts. Although Zeliff's measurements of *O. hubbardi* were based on 75 specimens, I found only two animals in three slides that were as large as his average. My averages were undoubtedly decreased by the inclusion of some very small animals, but random remeasurements justified the retention of my computations.

### **Oxymonas dimorpha** Connell

*Oxymonas dimorpha* Connell, 1930, Univ. Calif. Publ. Zoöl., 36:51, pl. 3.

*Type host.—Paraneotermes simplicicornis* Banks. California; Arizona.
T-316. (Xenosyntype slide TP-246:36.)

*Diagnosis.*—Body broadly pyriform or flask-shaped; length 120(17–190)$\mu$; width 100 (14–165)$\mu$; nucleus round, diameter 7.7(4–12.3)$\mu$ (Connell); chromatin in granular reticulum; karyosome round, surrounded by a halo, usually centrally situated; K/N = 1/3; H/K = 1/4; axostyle slender, projecting slightly beyond the posterior boundary of the body; pellicular symbionts consisting of heavy curved rods, 3.3$\mu$ in length; blepharoplasts, connecting rhizoplast, and flagella lost during the sessile stage.

Connell's minimum length included the short rostellum of the motile forms. The nucleus of *O. dimorpha* is small for so large an animal, its average size being close to that of the much smaller species *O. caudata, O. kirbyi, O. minor,* and *O. pediculosa* from *Rugitermes panamae.* The only other *Oxymonas* that is as large as *O. dimorpha* is *O. grandis,* from which it is differentiated in having a karyosome during interkinesis, a much smaller nucleus, and a slender axostyle. Connell's recognition of the diphasic existence of *Oxymonas* was a valuable contribution. In general, my study confirms his outline of the life cycle, except that nuclear division is not limited to animals that contain "volutin." His assumption that the spherules contain that substance is insufficiently supported, because the tests he cited are not specific and my tests for volutin (McClung, 1937) were negative.

My observation shows that Connell's "supporting fibrils" are actually fibrils from the recurvent part of the axostyle. His description of their origin confuses them with the expanded polymorphous nuclear matrix which maintains the connection between the nucleus and the new axostyle. Cleveland's studies (1934, 1935) and my own (1941) have discredited his report that the intra-

nuclear spindle originated from the karyosome. The fibers in Connell's figures 8 and 11 (1930) can scarcely be, as he has designated them, last remnants of the spindle, for there have been no instances in which the polar region of the spindle has been extruded from the nucleus or has been in juxtaposition with the blepharoplasts. In fact, the distance separating the pole of the spindle from the basal granules increases continuously as division progresses. Perhaps the disputed fibers are fibrils from the recurvent portion of the axostyle fortuitously placed in association with the basal granules. Connell questioned Kofoid and Swezy's (1926*a*) assumption that the evolutionary pattern of *Oxymonas* consisted of a tendency to increase the neuromotor units, and suggested instead that there was a tendency to increase the speed of reproduction. He also postulated the production of multinucleate animals from uninucleate forms by means of delayed cytokinesis.

### **Oxymonas grandis** Cleveland

*Oxymonas grandis* Cleveland, 1935, Biol. Bull., 69:54.

*Type host.—Neotermes dalbergiae* Kalshoven. Java.

T-4532. Bandjar. (Xenosyntype slide TP-3220:20.)
T-4564. Bandjar. (Xenosyntype slide TP-3225:3.)
T-4568. Bandjar. (Xenosyntype slide TP-3231:30C.)

*Additional host.—Neotermes tectonae* Dammerman. Java; Sumatra.

T-4563. Kateman. (Homosyntype slide TP-3223:3.)
T-4548. Tapos, Mt. Gedeh. (Homosyntype slide TP-3222:2.)
T-4531. Kateman. (Homosyntype slide TP-3215:45B.)

*Diagnosis.*—Body ovoid to ellipsoid, often irregular; length (median) 109(41–241)$\mu$; width (median) 36.4(15.3–78.3)$\mu$ (Cross); nucleus ovoid; length (median) 16(8.7–24.9)$\mu$; width (median) 12.6(8–20.7)$\mu$ (Cross); chromatin in granular reticulum; karyosome absent except during late reorganization of the nucleus following division; axostyle broad, resembling a scimitar in shape, projecting very slightly beyond the posterior boundary of the body; well-defined but delicately staining anterior, and loosely fibrous recurvent portions of the axostyle; pellicular symbionts consisting of rodlets averaging about 1.3$\mu$ in length, and a tuft of spirochaetes about 6$\mu$ long, situated above the shoulder and on the opposite side of the axostyle from that on which the nucleus lies.

The measurements were based on 51 animals. Cleveland used the arithmetical average and reported the length as 121(76–183)$\mu$ and the width as 52(31–79)$\mu$. His diameters for the nucleus were 21(20–23)$\mu$. It is noticeable that my minimums are lower and my maximums are higher. The absence of a karyosome in Cleveland's specimens and the presence of much smaller animals in mine support the belief that my material contained more immediately post-division animals than Cleveland's did. (See below, pp. 71–78, for a special study of the morphology of *O. grandis*.)

### **Oxymonas megakaryosoma** sp. nov.

*Type host.—Glyptotermes* sp. nov. Uganda.

T-2072. Kampala. (Syntype slides TP-2017:26, 2023:18, 2025:30.)
T-2090. Budongo Forest. (Xenosyntype slide TP-2028:13.)

*Diagnosis.*—Body spheroidal to ellipsoidal; length (median) 61.4(24.9–200.1)$\mu$; width (median) 26.4(9.3–82.3)$\mu$; nucleus ovoid; length (median) 10.7(6.7–17.3)$\mu$; width (median) 8.7(6.0–15.2)$\mu$; chromatin in granular reticulum; karyosome oval; length (median)

4(2.0–8.7)$\mu$; width (median) 3.3(1.3–6.0)$\mu$; surrounded by a halo (median) 1.3(0.0–3.3)$\mu$; excentric posteriorly; K/N = 1/2; H/K = 1/3; axostyle broad, resembling a scimitar in shape, the most posterior part consisting of a heavy fiber barely projecting beyond the cytoplasm; well-defined but delicately stained and loosely fibrous, recurvent and anterior portions of the axostyle; pellicular symbionts consisting of rodlets averaging about 1.3$\mu$ in length; binucleate animals not uncommon.

The most striking characteristic of *Oxymonas megakaryosoma* is the large, usually oval karyosome; but in 11 out of the 51 animals on which the measurements were based, the karyosome was round. A special study of the morphology of this species is given below, pages 78–79.

### Oxymonas notabilis sp. nov.

*Type host.*—*Neotermes howa* Wasmann. Madagascar.

T-4446. Manantantely. (Syntype slides TP-3187:20, 29, 36, 75.)

*Diagnosis.*—Body ovoid to slenderly ellipsoid to elongate irregular in shape; length (median) 59.3(27–125.4)$\mu$; width (median) 18.7(12.7–28.6)$\mu$; nucleus broadly ovoid; length (median) 9.3(7.3–14.0)$\mu$; width (median) 8.7(6.7–13.3)$\mu$; chromatin in granular reticulum; karyosome round, diameter (median) 2.7(2–4)$\mu$; excentric posteriorly; surrounded by a halo (median) 0.7(0.7–1.3)$\mu$; K/N = 1/3; H/K = 1/4; axostyle broad, irregularly pennant-shaped, much shorter than the body; not projecting beyond the posterior cytoplasm; shoulder of axostyle retracted toward the middle of the body; the anterior portion delicately stained; the recurvent portion deeply stained and composed of compact paralleling fibers; pellicular symbionts consisting of spirochaetes averaging about 7$\mu$ in length; and thick, slightly curved rods about 2.3$\mu$ in length; binucleate animals frequent, 4-, 5-, and 6-nucleate specimens not rare.

The measurements were based on 51 animals. The name *notabilis* was chosen because the deeply staining recurvent portion of the axostyle and the retracted shoulder of the axostyle have not been found in any other species of *Oxymonas*. A special study of the morphology of *O. notabilis* is given below, pages 79–80.

### Oxymonas di-undulata Nurse

*Oxymonas di-undulata* Nurse, 1945, Trans. Roy. Soc. New Zealand, 74:309.

*Type host.*—*Kalotermes brouni* Froggatt. New Zealand.

T-500. (Cleveland.) (Xenosyntype slides TP-500:20, 141, 247.)

*Diagnosis.*—Body ovoid to pyriform; length (15.3–38.2)$\mu$; width (12.2–27.5)$\mu$ (Nurse); length 21.2(7.8–40.5)$\mu$; width 9.0(6.1–22.3)$\mu$ (Cross); nucleus ovoid, chromatin in coarsely granular reticulum; length 5.6(3.9–8.4)$\mu$; width 3.9(2.8–7.2)$\mu$; karyosome round, excentric posteriorly; K/N = 1/3; H/K = 1/1 (usually); axostyle slender, broadened in region near nucleus, often shorter than the body, never projecting much beyond the posterior cytoplasm; delicately staining recurvent portion of the axostyle; matrix sleeves frequently present; pellicular symbionts consisting of cocci, infrequently present.

The report by Nurse (1945) that this oxymonad has two flagella, each of which is associated with an undulating membrane is not acceptable. My examination of smears made in New Zealand by Professor L. R. Cleveland from *Kalotermes brouni* revealed no evidence whatever of an undulating membrane and the blepharoplast-flagella complex is in complete agreement with that of the other oxymonads that I have studied. In her text figure 3, figure b, which supposedly depicts four undulating membranes, actually represents two matrix sleeves. (See above, p. 85 and pl. 13, figs. 81, 82, 85, 88, 89, 90.) These

structures are similar morphologically to Kofoid and Swezy's (1926*a*) axostyle sleeves.

Nurse used a weak solution of iodine alcohol as a vital stain. I did not find this technique at all reliable for studying the flagella of *Oxymonas minor* (Cross, 1941). Studies made with either darkfield illumination or fixed smears are preferable, but even Heidenhain's iron-haematoxylin stain is unsatisfactory for distinguishing the blepharoplast-flagella complex in *Oxymonas* unless the stain is much more intense than is ideal for other morphological investigations. The most satisfactory studies resulted from smears that had been fixed with Flemming's solution and stained with Regaud's haematoxylin. Evidently, Nurse saw only the blepharoplast which is situated adjacent to the axostyle and failed to find the second blepharoplast, which is closely associated with the axostyle's shoulder. It seems almost equally evident that her report of undulating membranes resulted from observation of the slashing movement of the recurvent portion of the axostyle in the endoplasm of living *Oxymonas*.

Nurse describes figure a in text figure 3, as having two nuclei ". . . but axostyle not completely divided, still single at the posterior end." This is another obvious error because in the entire subfamily of the Oxymonadinae, the axostyle disintegrates during early kinesis and forms *de novo*. This figure merely illustrates an instance where the posterior tips of the young axostyles chance to lie in close juxtaposition. My objections to her report of a retracting rostellum have already been stated (see above, p. 72).

*O. di-undulata* resembles *O. kirbyi* in the size and the shape of the body, in the character of the nucleus, in the tendency to produce matrix sleeves, and in having an axostyle that is commonly shorter than the body. The former differs from the latter in usually being more slender, in having a delicately staining recurvent portion of the axostyle, and in having cocci rather than rods as pellicular symbionts.

## Microrhopalodina Grassi and Foà

*Microrhopalodina* Grassi and Foà, 1911, R. C. Accad. Lincei, (5) vol. 20, 1 sem.: 730 (type species, *M. inflata*); Bernstein, 1928, Arch. Protistenk., 61:28; Kirby, 1928, Quart. J. Micr. Sci., 72:378; 1937, Univ. Calif. Publ. Zoöl., 41:205; Cleveland, 1934, Mem. Amer. Acad. Arts Sci., 17:304; Duboscq and Grassé, 1934, Arch. Zool. exp. gén., 75:626; De Mello and De Mello, 1944, Anais de Instituto de Medicina Tropical, 1:205.

*Proboscidiella* Kofoid and Swezy, 1926, Univ. Calif. Publ. Zoöl., 28:312; Bernstein, 1928, Arch. Protistenk., 61:30; Kirby, 1928, Quart. J. Micr. Sci., 72:361; 1937, Univ. Calif. Publ. Zoöl., 41:205; Lewis, 1933, Univ. Calif. Publ. Zoöl., 29:77.

*Diagnosis.*—Multinucleate Oxymonadinae; body varying from spheroidal to pyriform to irregular elongate forms; length 67 (23–165) $\mu$; width 40 (11–113) $\mu$; range in medians of nuclear numbers 5–13; range in extremes of nuclear numbers 1–50; nucleus spherical to ovoid, chromatin in granular reticulum or massed against the nuclear membrane, karyosome round or ovoid, surrounded by a halo; anterior corona formation of all, or almost all, of the nuclei during interkinesis; usually each nucleus in close association with the shoulder of one axostyle; axostyle slender, usually equal numerically to the nuclei, a recurvent portion never longer than the body and often much shorter, and an anterior portion; pellicular symbionts significant for the differentiation of all reported species.

*Microrhopalodina* was the first oxymonad to be reported and although their description was incomplete and lacked illustrations, Grassi and Foà (1911) recognized the complex, diphasic existence of the protozoan and correlated the alternation of an initial, uninucleate, motile stage, and a multinucleate, sessile period with the moulting habits of the host. Uninucleate animals have been reported from all the species of *Microrhopalodina* except *M. multinucleata,* but neither descriptions nor figures have been given illustrating their origin from multinucleates. Their presence in *M. inflata* was definitely verified because they are few in number and showed all of the characteristics of the multinucleate form. However, in *M. occidentis,* although I found a large number of uninucleate animals, only one specimen showed precisely the same characteristics of the multinucleate stage. The others did not have typical nuclei nor deeply staining anterior and recurvent portions of the axostyle. Immaturity might be the explanation of such nonconformance but would fail to explain the large number of uninucleate animals that are not contaminated with the pellicular spirochaetes of the presumed multinucleate "mother." It seems probable that *Kalotermes occidentis* harbors an additional uninucleate oxymonad and this would account for the excessive number of uninucleate animals reported by Lewis (1933). It is impossible, however, to verify this assumption, because no specimens of *Oxymonas* with definitely mature characters were found on the one slide available for study.

A parabasal that was either very difficult or impossible to see was reported by Grassi and Foà (1911) and the genus was consequently placed among the calonymphids. Neither Duboscq and Grassé (1934) nor I have been able to find this organelle in the type species from *Kalotermes flavicollis.* Kirby (1928) and Lewis (1933) reported aggregations of small, deep-staining granules above the nuclei in *M. multinucleata* (= *M. kofoidi*) and in *M. occidentis.* Their locus was that of a parabasal body, but they were not consistently present and were not accepted as true parabasals. Kirby (1928) also commented on similar granules appearing in *Oxymonas clevelandi.* Probably, the granules were actually the short chains of cocci which constantly localize at the shoulder of the axostyle in that species and produce a "beaded" appearance. The illustrations in *M. multinucleata* (Kirby, 1928) and in *M. occidentis* (Lewis, 1933) support this view. In *Barroella coronaria* two or three animals were seen in which only a small number of axostyles were infested, and cocci have recently been seen in the same position in a uninucleate specimen of *M. inflata* and in several specimens of *M. multinucleata.* Possibly Grassi and Foà (1911) used material in which a similar, constantly localized infestation of cocci occurred fortuitously oftener than in the material obtained by subsequent observers, and mistook the granular aggregations for parabasal bodies.

Duboscq and Grassé (1934) considered that the absence of a parabasal in *Microrhopalodina* excluded it from the Calonymphidae and prevented the inclusion in *Microrhopalodina* of *Opisthomitus avicularis* although it is also a multinucleate and leads a diphasic existence. Observations on smears and sections which were not obtainable previously confirm that viewpoint and there is an additional important objection to my earlier suggestion (1941)

that *Opisthomitus* was possibly an *Oxymonas,* namely, that the nucleus of the former genus is entirely different from that of the oxymonads.

Kirby (1928) suggested the possible synonymy of *Microrhopalodina* and *Proboscidiella* but retained the latter because *Microrhopalodina* had been insufficiently described. Duboscq and Grassé (1934) considered that *Proboscidiella* was a distinct genus because ". . . leurs axostyles élargis et qui peuvent avoir à la fois rostre et flagelles." Instead, they surmised that *Kirbyella* (=*Barroella*) was in synonymy with *Microrhopalodina* because it had the ". . . même forme des stades sans flagelles (les seuls vus par Zeliff), même disposition générale des axostyles et des fibres rostrales." Comparison of the disputed forms does not support Duboscq and Grassé's contention that the axostyle of *Microrhopalodina* is more slender; and separation from *Proboscidiella* because of the retention or nonretention of the flagella during the sessile period does not seem justified. Kirby (1928) reported that in living *M. multinucleata,* the presence or absence of flagella on rostellate animals was variable. Cleveland (1934) placed *Proboscidiella* in synonymy with *Microrhopalodina,* but gave no explanation for doing so. My reasons for agreeing with him and for disagreeing with Duboscq and Grassé's merging of *Kirbyella* (=*Barroella*) and *Microrhopalodina* have been given on page 96.

Kofoid and Swezy (1926*b*) described *Microrhopalodina* as having a primary and two secondary flagella, and De Mello and De Mello (1944) reported only two. Neither of these descriptions is in accord with those of other species. Since it was evident that the specimens of De Mello and De Mello (1944) had been insufficiently stained to delineate properly the axostyle and its relationships, it is logical to assume that there would be an even less satisfactory delineation of the blepharoplast and flagella complex which is always more difficult to trace. This being true, it seems justifiable to disregard De Mello and De Mello's report of only one flagellum for each blepharoplast. Additional support is given this decision by the fact that De Mello and De Mello (1944) have shown two flagella originating from one blepharoplast in two instances in their plate I.

It is true that in *Barroella coronaria* there were many specimens with three flagella, but two of the three occurred indiscriminately on either of the two blepharoplasts. Apparently, one flagellum is frequently destroyed, and a total of four flagella with two from each of the blepharoplasts is normally present in unmutilated specimens. The latter condition is so nearly uniform throughout the Oxymonadinae that it is accepted in this paper as a diagnostic character of the subfamily, and variations have been listed as questionable. Kirby's (1928) sketches of the axostyle-blepharoplast-flagella complex are probably typical for the genus. They agree with the incomplete description of the combination in *M. inflata* (Duboscq and Grassé, 1934) and with the condition pictured by Lewis (pl. 9, fig. 15, 1933) for *M. occidentis,* and with my observations on a binucleate animal of the latter species. They are also very similar to the combinations in *Oxymonas* (pl. 5, fig. 18; pl. 9, fig. 39) and in *Barroella* (pl. 14, fig. 100).

Because the morphology of the "retractor fibers" as they were described by

Kofoid and Swezy (1926*b*) and of the "cytoplasmic fibers" described by Kirby (1928) and Lewis (1933) is identical with that of the recurvent portion of the axostyle in *Microrhopalodina,* both of the above-named terms have been discarded. Objections and arguments regarding Kofoid and Swezy's assumption that the rostellum is "extensile and retractile" and that fission occurs without an associated mitosis have been given on pages 72 and 84.

Kirby (1928) made the first detailed report of mitosis in the oxymonads and described and pictured the posterior migration of the nucleus during kinesis in *M. multinucleata.* Janicki's (1915) illustrations implied this migration, but there is nothing in his description to indicate that the seemingly haphazard change in nuclear position is an essential step in the mitotic pattern. Duboscq and Grassé (1934) were evidently still unaware of the importance of the concept of nuclear migration in interpreting division phenomena in the oxymonads when they concluded a footnote describing such nuclear behavior by saying, "S'agit-il là d'individus en voie de dégénerescence ou d'un stade dont nous ne voyons pas la place, nous ne pouvons le dire."

The spelling of the type species name was emended from *enflata,* and a fairly comprehensive description of the diphasic morphology of *Microrhopalodina inflata* was presented by Duboscq and Grassé (1934). Kirby (1928) and Lewis (1933) were doubtful of the cyst formation reported by Kofoid and Swezy (1926*b*). Observations on other oxymonads support their objection that the so-called "cysts" were actually spherical animals in which the peripheral ectoplasm was wider and more highly vacuolated than usual. Mitosis was reported briefly by Lewis (1933) and Duboscq and Grassé (1934), but nothing new was added to Kirby's (1928) earlier description. Structures like the "tracheal tubes" described by Lewis are found in the intestinal contents of the termite and infrequently in the cytoplasm of an oxymonad, but they are not identical with the tubular matrix sleeves and, contrary to Lewis's report, they retain the same yellow color when inside the protozoan.

**Microrhopalodina inflata** Grassi and Foà, emend. Duboscq and Grassé

*Microrhopalodina enflata* Grassi and Foà, 1911, R. C. Accad. Lincei, (5) vol. 20, 1 sem.:739.

*Microrhopalodina inflata* Duboscq and Grassé, 1934, Arch. Zool. exp. gén., 75:626.

*Type host.—Kalotermes flavicollis* Fabricius. Europe.

T-323. (Xenosyntype slide TP-197:7.)

*Diagnosis.*—Body varying from spheroidal to pyriform to irregular elongate forms; length 13–90$\mu$; nuclear numbers "un peu moins" 12 (1–"plus de 50") (Duboscq and Grassé); nucleus spherical to ovoid, chromatin in granular reticulum; karyosome round, surrounded by a halo, slightly excentric; irregular anterior corona formation of nuclei, not all nuclei being closely associated with shoulder of axostyle; K/N = 1/3; H/K = 1/3 to 1/1; axostyles slender, extending full length of body; anterior and recurvent portions of axostyle deeply stained; pellicular symbionts consisting of short rods, 2–3$\mu$ in length, and spirochaetes about 7$\mu$ long (Cross) or 20$\mu$ long and shorter (Duboscq and Grassé).

Most of the account of Grassi and Foà (1911) consisted of a description of the sessile and motile stages which comprise the life cycle. They erred in reporting the presence of only one blepharoplast in the motile stage, and made no attempt to enumerate the flagella. In an amplification of their report,

Duboscq and Grassé (1934) described an eight-nucleate animal with a total of 32 flagella in groups of two from each blepharoplast. From this it may be assumed that two blepharoplasts were associated with each nucleus. Observation has supported the assumption and supplied the additional facts that the two large blepharoplasts were each composed of two closely appressed spherical granules, and that one of the blepharoplasts was joined directly to the shoulder of the axostyle and less directly to the other blepharoplast by means of a short fiber. In agreement with both of the earlier reports, no blepharoplasts nor basal granules were found in the sessile stage.

*M. occidentis* is the only other species that has deep-staining anterior and recurvent portions of the axostyle, but the ratios of K/N and H/K are quite different in *M. inflata,* and the axostyle is broader and less frequently as long as the body. Duboscq and Grassé (1934) commented on the peculiar spiral course of the rostellar fibers in the latter species and this behavior is much less pronounced in *M. occidentis.* There are no short rods among the pellicular spirochaetes of the latter species, and the spirochaetes themselves are much shorter than in *M. inflata.*

### **Microrhopalodina multinucleata** (Kofoid and Swezy) comb. nov.

*Proboscidiella multinucleata* Kofoid and Swezy, 1926, Univ. Calif. Publ. Zoöl., 28:301.
*Proboscidiella kofoidi* Kirby, 1928, Quart. J. Micr. Sci., 72:361.

*Type host.—Cryptotermes dudleyi* Banks. Philippine Islands; Panama. T-239. Balboa, Canal Zone. (Xenosyntype slide TP-159:4.)

*Diagnosis.*—Body spherical to pyriform; length 72(25–160)$\mu$; width 25–113$\mu$; nuclear numbers 8(1–34) (Kofoid and Swezy); length 66(23–165)$\mu$; width 46(12–100)$\mu$; nuclear numbers (median) 8(2–26)$\mu$ (Kirby); nucleus spherical to ovoidal, chromatin in granular reticulum; karyosome round to ovoid, surrounded by a halo, excentric posteriorly; anterior corona formation of nuclei during interkinesis, each nucleus being closely associated with the shoulder of an axostyle; K/N = 1/3; H/K = 1/3; axostyle slender, usually not more than ½ to ⅔ of the length of the body; pellicular symbionts consisting of a coat of short curved rods.

*Microrhopalodina multinucleata* is distinguished readily from *M. inflata* and *M. occidentis* by its delicately stained anterior and recurvent portions of the axostyle. It is distinguished from *M. hofmanni* by the close association of the nucleus with the axostyle shoulders and by axostyles that are usually shorter than the body.

Emerson (personal communication to Professor Kirby, 1945) has determined that *Planocryptotermes nocens* Light from the Philippine Islands is in synonymy with *Cryptotermes dudleyi* Banks. Therefore, the hosts of the species reported earlier as *P. multinucleata* Kofoid and Swezy (1926*b*) and *P. kofoidi* Kirby (1928) are the same and this constitutes a potent argument for the synonymy of the oxymonads. The study of *M. multinucleata* (Kofoid and Swezy) has been confined to their sketches and description because slides from *Cryptotermes dudleyi* from the Philippines have not been available. Kirby's report of *P. kofoidi* has been verified by me from specimens in smears from *C. dudleyi* from Panama. He distinguished *P. kofoidi* from *P. multinucleata* because of its proportionately larger karyosome, because of its differ-

ent flagella-blepharoplast complex, and because the pellicular symbionts differed from the long, straight rods that were pictured by Kofoid and Swezy (1926). The last-named difference has been disregarded because their sketch was highly stylized and Kirby's description of short, curved rods has been used in the diagnosis. Kirby's studies with darkfield illumination definitely established the number of flagella as being two for each of the two blepharoplasts and the report of three by Kofoid and Swezy (1926*b*) has been further discredited by my observations on *Barroella coronaria* (p. 116). The importance of the ratio K/N as a diagnostic character was not recognized by Kofoid and Swezy (1926*b*), but an estimate based on their measurements indicated that 1/5 was the maximum in *P. multinucleata*. However, where measurements were made of their sketches, there were a number of specimens where the ratio was 1/4 and a few where it was 1/3. Because of this discrepancy between the estimate and the sketches, Kirby's figures have been accepted in the diagnosis. It is probable that if sufficient specimens of *P. multinucleata* Kofoid and Swezy were investigated, the fibers of the recurvent portion of the axostyle (called "retractor fibers" by Kofoid and Swezy and "cytoplasmic fibers" by Kirby) would be found to extend "longitudinally throughout the endoplasm" and not to be limited to the peripheral region, and that sometimes these fibers would be almost as long as the body.

In addition to describing *M. multinucleata,* Kofoid and Swezy (1926*b*) discussed its importance in illustrating an intermediate step in the evolution from a simple cellular state to a multicellular somatella. They accepted *Oxymonas* as representing the initial stage in the process with the rostellum preserving the "unitary nature" in *Microrhopalodina* in spite of the multiplication of nuclei and neuromotor units. In *M. multinucleata,* Kofoid and Swezy again emphasized the importance of the neuromotor system because of their belief that it controlled the extension and retraction of the rostellum. My observations (above, p. 72) have not supported their assumption. They have attributed the presence of an axostyle that projects beyond the posterior boundary of the body in one of their specimens (pl. 31, fig. 2, Kofoid and Swezy, 1926*b*) to the retraction of the rostellum, but it can be more simply explained as a recently divided animal in which the growth adaptation of a lengthy rostellum has not as yet taken place and in which the tips of the axostyles protrude because of a fortuitously meager apportionment of cytoplasm during fission. According to the latter viewpoint, the protruding axostyles represent a chance and transient condition of immaturity and do not violate the diagnostic stipulation in *Microrhopalodina* that limits the length of the axostyle to that of the body.

Kirby (1928) presumed that the rhizoplasts which were directed posteriorly from the blepharoplasts toward the nucleus might maintain the latter organelle in its position. Even in larger oxymonads, it frequently happens that only the fibrous-appearing boundaries of the interkinetic nuclear matrix which holds the nucleus to the shoulder of the axostyle can be observed; and it seems altogether possible that the posteriorly directed rhizoplasts described for the smaller animals represent these boundaries.

**Microrhopalodina occidentis** (Lewis) comb. nov.

*Proboscidiella occidentis* Lewis, 1933, Univ. Calif. Publ. Zoöl., 39:77.

*Type host.*—*Kalotermes occidentis* Walker. Mexico.

T-356. Lower California. (Xenosyntype slide TP-317:14.)

*Diagnosis.*—Body spheroidal to pyriform to irregular elongate; length 64(26–133)$\mu$; width 36(11–80)$\mu$; nuclear numbers 5 (median) (1–17) (Lewis); nucleus spherical to ovoid; chromatin granules massed against the nuclear membrane; karyosome round, surrounded by a halo, slightly excentric; anterior corona formation of nuclei, each nucleus closely associated with the shoulder of an axostyle; K/N = 1/4 (Lewis); H/K = 1/1; axostyle slender, often extending the full length of the body; anterior and recurvent portions of axostyle deeply stained; pellicular symbionts consisting of spirochaetes about 5$\mu$ long (Cross).

Except for *Microrhopalodina inflata, M. occidentis* is easily differentiated from the other species in the genus because the anterior and recurvent portions of the axostyle stain deeply, and the characters that distinguish them from one another have been discussed with the first-named species. In differentiating *M. occidentis,* Lewis (1933) compared the sizes of the karyosomes relative to their respective nuclei. This is the same as the procedure indicated briefly by the ratio K/N in this paper. He described both the axostyle and the blepharoplast bar as being broader than in *M. multinucleata* and in *M. kofoidi,* and gave the length of the flagella as 18 and 60 microns, respectively, for the last-named species, and 40 microns for *M. occidentis.* The flagella of *M. multinucleata* were reported as being dissimilar from the others because of their inequality in thickness. A rostellum was reported by Lewis (1933) that was 423$\mu$ in length. It was seven times as long as the specimen and is the longest rostellum recorded for the Oxymonadinae. Observations confirmed his statement that the two blepharoplasts were approximately the same in size, but Lewis did not discover that they are each composed of two closely appressed spherical granules and that the flagella originate from the distal member of the pair. Blepharoplasts were present in mature animals with long rostella. Lewis's report that the pellicular symbionts of *M. multinucleata* and *M. occidentis* are different, can be extended to include *M. inflata* as well.

**Microrhopalodina hofmanni** (De Mello and De Mello) comb. nov.

*Proboscidiella Hofmanni* De Mello and De Mello, 1944, Anais de Instituto de Medicina Tropical, vol. 1, fasc. 2, 211. Lisbon, Portugal.

*Type host.*—Incompletely described as "Indian Cryptotermes."

*Diagnosis.*—Body spherical to ellipsoidal; length 27(12–54)$\mu$; width 20(11–56)$\mu$; nuclear numbers 4(2–14) (De Mello and De Mello); nucleus ovoid, chromatin in granular reticulum; karyosome round, surrounded by a halo, excentric; irregular, anterior corona formation of nuclei; nuclear number not the same as the number of axostyles; K/N and H/K not recorded; axostyle slender and deeply stained, extending the full length of the body; the anterior and recurvent portions of the axostyle delicately stained; pellicular symbionts lacking in 99 per cent of the specimens.

The medians in the above measurements were derived from figures given by De Mello and De Mello. *M. hofmanni* is very much smaller than any other described *Microrhopalodina.* This species is like *M. inflata* and unlike *M. multinucleata* and *M. occidentis* because the formation of the nuclear corona is

irregular and because its axostyles extend practically the full length of the body; but *M. hofmanni* resembles *M. multinucleata* and differs from both *M. inflata* and *M. occidentis* in having delicately staining anterior and recurvent portions of the axostyle.

Both the sketches and the descriptions by De Mello and De Mello indicate that their specimens have been insufficiently stained to show the true nature and relationship of the axostyles. The fact that there is a lack of close association between the nuclei and the axostyles in the interkinetic stage does not prove, as De Mello and De Mello have assumed, that there is no relationship between the two organelles. In *O. notabilis* (text fig. A, fig. 4) an even greater separation between the nucleus and the axostyle is present in interkinesis, but during mitosis the association is equally as close as in the other oxymonads. De Mello and De Mello state that they have pictured all of the mitotic figures that they found. Very little information on mitotic behavior can be gained from so small a number, but none of their specimens deviated from the scheme of division described for oxymonads on page 92.

Since diploid arrangements of chromatin granules are of common occurrence in the early prophase nucleus of the Oxymonadinae, the diploid granules which De Mello and De Mello have reported as dividing centrioles cannot be accepted as such. The scarcity of dumbbell-shaped karyosomes is undoubtedly correlated with the scarcity of mitotic figures because division of that organelle (see above, p. 88) takes place either during the prophase or late reorganization in all the subfamily.

The "cerclet of anterior flagella" which is described by De Mello and De Mello is evidently a schematized representation of disrupted fibers of the holdfast. These would not be present, as they have reported truly, on sessile forms; but it is unlikely that the number of fibrils would be 6 or any other constant number.

## Barroella Zeliff

*Kirbyella* Zeliff, 1930, Amer. J. Hyg., 11:740 (type species, *K. zeteki*); Cleveland, 1934, Mem. Amer. Acad. Arts Sci., 17:303; Duboscq and Grassé, 1934, Arch. Zool. exp. gén. 75:635; Kirby, 1937, Univ. Calif. Publ. Zoöl., 41:205; De Mello and De Mello, 1944, Anais de Instituto de Medicina Tropical, 1:217.

*Barroella* Zeliff, 1944, J. Parasitol., 30:275.

*Diagnosis.*—Multinucleate Oxymonadinae; body varying from subspheroidal to pyriform to irregular elongate forms; length (median) 75(27–224)$\mu$; width 34(11–80)$\mu$; range in medians of nuclear numbers 7–24; range in extremes of nuclear numbers 2–114; nuclei spherical to ovoid, chromatin massed against nuclear membrane or in granular reticulum; karyosome surrounded by a halo; nuclei scattered throughout the body in mature animal; axostyles slender with delicately staining anterior portion and deeply staining recurvent portion, tortuously looped, much longer than the body; nuclei and axostyles rarely equal in number; immature animals similar to *Microrhopalodina;* with solitary anterior corona formation of nuclei; axostyles usually shorter than the body; axostyles and nuclei rarely equal in number; the immature animals originating by serial budding from larger animals, distinguished by multiple corona formation; each corona representing a unit which is similar to *Microrhopalodina* in the structure and arrangement of its component axostyles and nuclei and which is the end product of a modified anterior post-telophase migration of the nucleus; pellicular symbionts significant for differentiation of species.

Zeliff's (1930) definition of the genus did not include recognition of the immature subspheroidal forms nor the irregular, elongate shapes. Studies of mitosis show that the origin of the axostyle is exactly the same as that of *Oxymonas* and it is only the heavily stained recurvent portion that originates from a "common point at the tip of the rostellum or at the tip of the organism when this organelle is absent." The axostyles are not always more numerous than the nuclei, as he stated, but may be the same or less in number. Throughout the subfamily, the size of the karyosome proportionate to that of the nucleus has served as a means of differentiating species, and this is too narrowly limiting a character to serve for distinguishing genera as Zeliff (1930) has used it in *Barroella.*

Duboscq and Grassé (1934) surmised that *Kirbyella* (= *Barroella*) should be placed in synonymy with *Microrhopalodina,* but I have retained the distinction of the two genera for the reasons stated on page 96. The granular cytoplasm, the yellowish colored spherules, the wood particles enclosed in vacuoles, the blepharoplast-flagella complex and the variability in the shape of the body and of the rostellum in the multinucleate *Barroella* find their counterparts in the uninucleate *Oxymonas.* In both genera, each nucleus is associated with two blepharoplasts, each of which supports a pair of flagella. In *Barroella,* the presence of flagella is limited to the motile period, but that limitation is also evident in *O. dimorpha.* The overgrowth in length of the prominent, deeply stained, recurvent portion of the axostyle in *Barroella* is similar to the development of *O. notabilis.* In both, the customarily close association of the nucleus with the shoulder of the axostyle is lost. In *O. notabilis,* the nucleus is situated at the base of the rostellum and the shoulder is in the mid-region of the body. In *Barroella,* the positions are reversed. By these comparisons, it is demonstrated that the seemingly haphazard arrangement of the components of *Barroella* fits readily into the orderly, morphological pattern established for the uninucleate *Oxymonas.*

Since De Mello and De Mello (1944) only quoted Zeliff (1930*b*) and added nothing to the latter's statements, comment on their report is unnecessary.

### Barroella zeteki Zeliff

*Kirbyella zeteki* Zeliff, 1930, Amer. J. Hyg., 11:740.

*Barroella zeteki* Zeliff, 1944, J. Parasitol., 30:275.

*Type host.*—*Calcaritermes brevicollis* Banks. Panama.
T-121. Barro Colorado. (Xenosyntype slide TP-57:5.)

*Diagnosis.*—Body spheroidal to pyriform to irregular elongate in shape; length 56(35–100)$\mu$; width 30(15–60)$\mu$ (Zeliff); nuclear numbers 8(2–12) (Cross); nucleus round, diameter 6$\mu$ (Zeliff); chromatin massed in heavy granules against the nuclear membrane; karyosome round, surrounded by a halo, centrally situated; in mature form, nuclei never closely associated with axostyle but scattered throughout the body; K/N = 2/3; H/K = 1/3; axostyle slender, as long as body; recurvent portion of axostyle long and deeply stained; pellicular symbionts consisting of very small rods, often absent or difficult to see.

The nuclei of *Barroella zeteki* and *B. coronaria* are very different. In the former, large granules of chromatin are massed against the nuclear membrane and the ratio of K/N = 2/3. In the latter the granules appear in a reticulum

and the ratio of K/N is 1/3. The maximum number of nuclei reported for *B. zeteki* is only one-half the median number of nuclei for *B. coronaria.* There is a correspondingly smaller number of axostyles in the former and the recurvent portions of the axostyle are less constantly looped and contorted. Its pellicular symbionts are very small rods, and those of *B. coronaria* are long spirochaetes and curved rods.

Moundlike rostella are usually present on the subspheroidal, *Microrhopalodina*-like forms. There is little question but that these originate by serial budding, as has been illustrated for *B. coronaria* (pl. 15, figs. 102–105). One specimen of this form was found in which the blepharoplasts could be distinguished with difficulty, but they occurred in the number and position characteristic of oxymonads. In agreement with what was reported by Zeliff (1930*b*), no blepharoplasts were seen in the mature forms after the nuclei had left their earlier anterior position.

A uninucleate oxymonad, *O. ovata,* has also been reported as occurring in *Calcaritermes brevicollis* and its nucleus is very similar to that of *B. zeteki.* It is not illogical that uninucleate forms of *B. zeteki* might originate by budding in the same manner as the *Microrhopalodina*-like forms do. The axostyle in *O. ovata* is not the complex organelle seen in *B. zeteki,* but this might be attributed to immaturity. The pellicular symbionts of *O. ovata* occur occasionally as small patches of large diplococci. There is some uncertainty about the symbionts of *B. zeteki* because the stain was unfavorable for their study. Apparently, this species is covered with very small rods, and, infrequently, large mono- and diplococci occurred as cytoplasmic parasites. It seems desirable to maintain the uninucleate form, *O. ovata,* as a distinct species either until division forms have been discovered in which uninucleate animals similar to *O. ovata* are derived from *Barroella* or until a sufficient number of intermediate forms have been found to bridge the differences now existing between them. Either event would prove the immaturity of *O. ovata* and place it in synonymy with *B. zeteki.*

### **Barroella coronaria** sp. nov.

*Type host.*—*Neotermes howa* var. *mauritiana* Sjöstedt, Mauritius.

T-4522. (Syntype slides TP-3201:11, 59, 85, 94E.)

*Diagnosis.*—Multinucleate; body spheroidal to ellipsoidal, often irregular; length (median) 94.7(27–223.7)$\mu$; width (median) 37(10.7–80.3)$\mu$; nuclear numbers (median) 24(4–114); nucleus spheroidal; diameter (median) 4.7(3.3–6.7)$\mu$; chromatin in granular reticulum; karyosome round, diameter (median) 1.3(0.67–2.3)$\mu$; slightly excentric; surrounded by a halo; K/N = 1/3; H/K = 1/2; axostyle slender, anterior portion delicately stained; recurvent portion deeply stained, long, and contorted; shoulders of axostyles not in close association with nuclei; the numbers of axostyles infrequently equivalent to that of the nuclei; pellicular symbionts consisting of thick, curved rods, about 7$\mu$ long, and spirochaetes mostly about 7$\mu$ in length, but some over 9$\mu$ long.

The measurements are based on 51 animals. A special study of the morphology of *B. coronaria* is given on pages 81–82, and its differentiation from *B. zeteki* is discussed with the latter species.

## SUMMARY

1. A systematic account of the flagellate subfamily Oxymonadinae is presented. *Proboscidiella* Kofoid and Swezy is placed in synonymy with *Microrhopalodina* Grassi and Foà. The flagellate of *Rugitermes panamae* that was described as *Oxymonas panamae* by Zeliff (1930) is considered to be *O. pediculosa* Kofoid and Swezy. The flagellate of *Kalotermes perezi* that was incorrectly identified with *O. panamae* by Zeliff (1930) is given a new name, *O. caudata*. *O. ovata* Zeliff of *Calcaritermes emarginicollis* is separated from *O. ovata* of *Calcaritermes brevicollis* and is described as a new species, *O. rotunda*. *Proboscidiella kofoidi* Kirby is considered to be the same as *Microrhopalodina multinucleata* (Kofoid and Swezy).

2. Observations have been made on twenty-three of the twenty-seven species that are listed. Most of Zeliff's species are retained, but for the diagnoses it has been necessary to give additional characteristics and to substitute less variable ones for those he used.

3. Detailed cytological studies were made of *O. grandis* Cleveland, *O. megakaryosoma*, sp. nov., *O. notabilis*, sp. nov., and *Barroella coronaria* sp. nov.

4. The holdfast is composed of minute fibrils which anchor the animal to the chitinous intima of the gut in much the same fashion as small rootlets twine among the interstices of a roughened rock surface. The rostellum is not an "extensile and retractile" organelle, but is long or short in a passive growth adaptation to the requirements of its environment.

5. Tests for volutin in the cytoplasmic spherules were negative. The Feulgen test gave positive reactions for the karyosome and negative reactions for the cytoplasmic spherules, for the intranuclear spindle, and for the cytoplasmic parasites, *Sphaerita*.

6. In *Oxymonas*, the nuclear matrix typically maintains a continuous association of the nucleus and the axostyle from the latter's origin to its dissolution. In *Barroella* the association is maintained only until the close of the corona-forming period. Since there is evidence that a modification of the amorphous nuclear matrix is identical with the structures formerly called "axostyle sleeves," the term "matrix sleeve" has been preferred.

7. The mitotic pattern characteristic of the Oxymonadinae has been outlined on the basis of mitotic studies of *O. grandis* Cleveland, *O. megakaryosoma* sp. nov., *O. notabilis*, sp. nov., and *Barroella coronaria* sp. nov.

8. The intranuclear spindle is not a bar. Approximately paralleling fibers are added to an originally short, slender strand. The fibers are increased in number, compacted, and lengthened until a heavy, fibrous, cablelike structure results in the telophase. In company with the chromosomal fibers, it forms a bulging, cylindrical, intranuclear, achromatic figure. The intranuclear spindle either stains faintly or not at all with Delafield's haematoxylin.

9. Cleveland's report of the absence in *O. grandis* of a karyosome requires modification, because it is present transiently, during the late telophase and late reorganization of the daughter nuclei. The intranuclear spindle does not originate from the karyosome, for that organelle disintegrates either at the

close of late reorganization of the nuclei or during the prophase. The presence of more than one karyosome is an early aspect of its disintegration. An aggregation of chromatin granules around the degenerating intranuclear spindle in the telophase forms the new karyosome, and since the response of the karyosome to the Feulgen reaction is positive, it is unlikely that the spindle contributes much, if anything, to its formation.

10. The anterior post-telophase migration of the nucleus, preceding plasmotomy, that was reported for *Oxymonas minor* has been observed in *O. grandis, O. megakaryosoma,* and *O. notabilis,* and was figured previously for *Microrhopalodina* as well. In uninucleate oxymonads, this return of the nucleus to its interkinetic position before cytokinesis precludes the assumption of transverse fission which is otherwise implied by later division figures. Consequently, the presence of longitudinal cytokinesis which is an essential characteristic of the Mastigophora is established. In *Microrhopalodina* the illustrations supposedly demonstrated cytoplasmic fission without an accompanying mitosis. The fact of anterior migration of the nucleus, however, explains this seemingly aberrant behavior as normal cytokinesis which has only been delayed until after the daughter nuclei, which resulted from division in the posterior region of the body, have returned to their former interkinetic position at the base of the rostellum. In the normal cytoplasmic fission of uninucleate oxymonads, the delay results in animals that appear temporarily as binucleate specimens, and it is logical to suppose that true binucleate oxymonads can result if the period is extended indefinitely. From this it may be seen that Connell's hypothesis that multinucleate oxymonads originated by means of delayed cytokinesis in uninucleate animals assumed in addition the presence of nuclear migration. The actual existence of anterior post-telophase migration of the nucleus preceding plasmotomy makes the assumption unnecessary and consequently increases the plausibility of the theory.

11. The spectacular localization of numerous coronas of nuclei throughout the body of *Barroella coronaria* is the result of a modified anterior post-telophase migration of the nuclei. A tendency to migration following the telophase is retained, but the excessive number of nuclei makes the formation of a solitary corona in the limited region at the base of the rostellum impossible. Multiple corona formation at numerous points throughout the body is substituted, and the variation serves by force of numbers to accentuate the pattern of behavior and to strengthen the postulate that cytoplasmic division is preceded by an anterior post-telophase migration of the daughter nuclei in all of the Oxymonadinae.

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# PLATES

All drawings were made with a camera lucida and reduced by one-third in the reproductions. The sketches were made from smears except where it is stated that they were made from sections. Unless otherwise stated, all sketches of entire animals are shown at a magnification of × 550 and all detail drawings of nuclei at × 1540. J. B. Cross, delineator.

Abbreviations for methods of preparation: C, Champy's fluid; D, Delafield's haematoxylin; E, eosin; F, acid fuchsin; Fl, Flemming's fluid; H, Heidenhain's haematoxylin; Holl, Hollande's solution; R, Regaud's haematoxylin; S, Schaudinn's fluid; Z, Zenker's fluid.

## PLATE 3

*Oxymonas grandis*

Fig. 1. Prophase: detail of nucleus in fig. 2.

Fig. 2. Entire: prophase; nucleus migrating toward posterior; recurvent portion of axostyle forming a loop in cytoplasmic protuberance. From *Neotermes dalbergiae*. Holl-H.

Fig. 3. Prophase: early spindle. From *N. tectonae*. S-H.

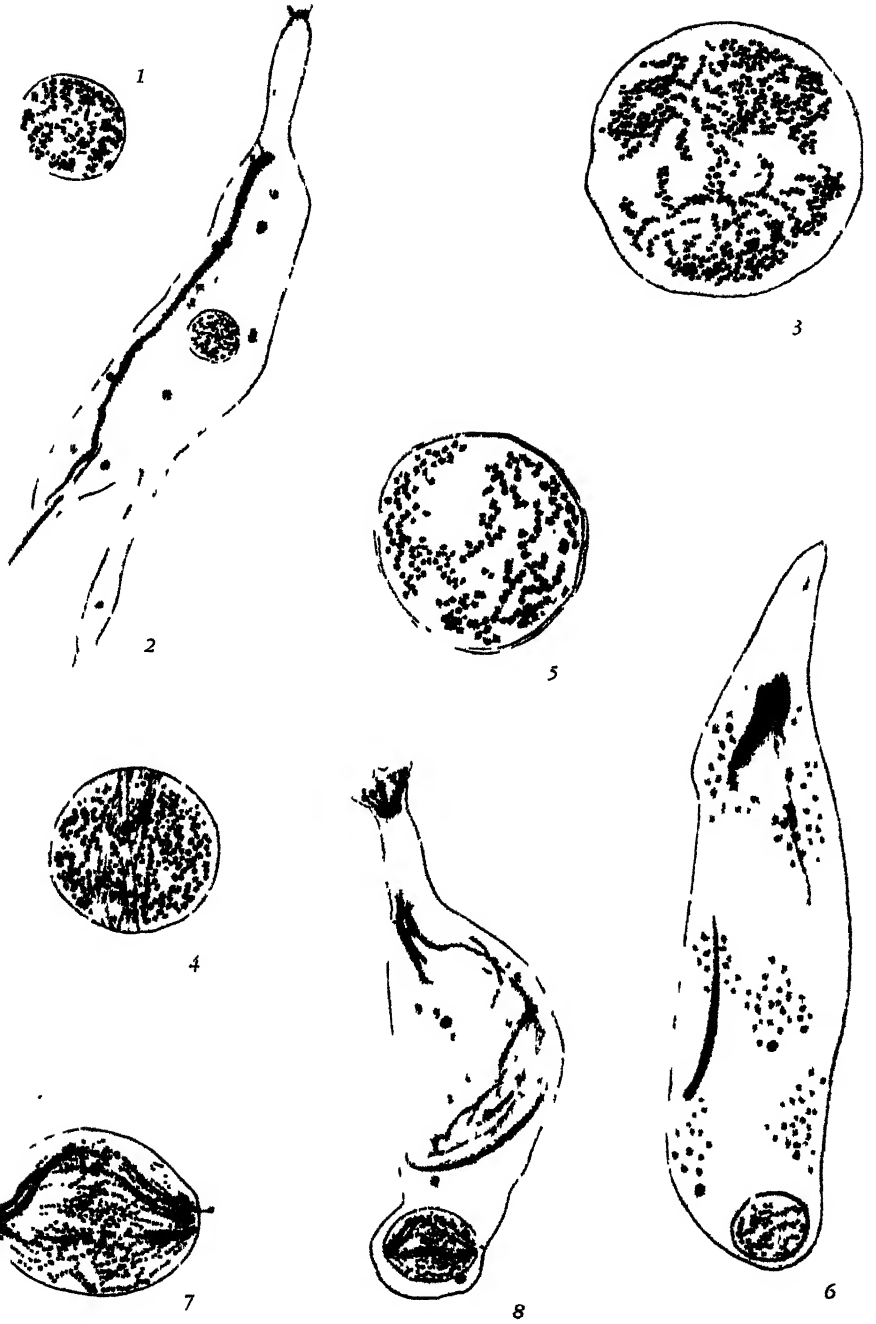
Fig. 4. Prophase: early spindle. From *N. tectonae*. S-H.

Fig. 5. Prophase: detail of nucleus in fig. 6; early spindle and concentric membranes on nucleus. From *N. dalbergiae*.

Fig. 6. Entire: prophase; nucleus situated posteriorly; axostyle degenerating. S-H-F.

Fig. 7. Late prophase: early intranuclear spindle with tubular poles; one blepharoplast. From *N. dalbergiae*. Holl-H.

Fig. 8. Entire for fig. 7: degenerating axostyle and holdfast; nucleus perpendicular to earlier orientation.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## PLATE 4

*Oxymonas grandis*

Fig 9 Late prophase early axostyles From *N tectonae* C H

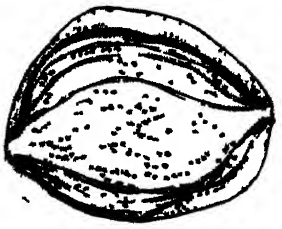
Fig 10 Metaphase chromatin in equatorial girdle From *V dalbergieae* Fl R

Figs 11 14 1 and 12 are consecutive anaphase stages Notice the anterior portion of the axostyles

Figs 11 1? Compare fig 1? with fig 72 From *V dalbergiae* Holl H

Fig 12 Much curved ribbonlike portions of spindle Compare with fig 51 From *V tectonae* Section Holl H

Fig 14 Notice the definite attachment of the nuclear matrix to the axostyle From *V tectonae* S H



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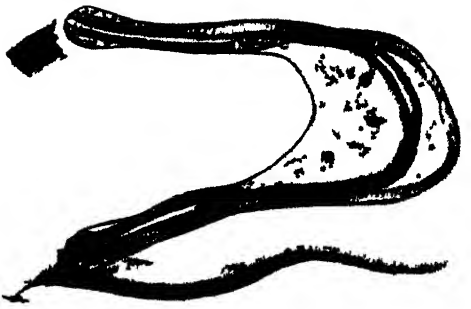

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## PLATE 5

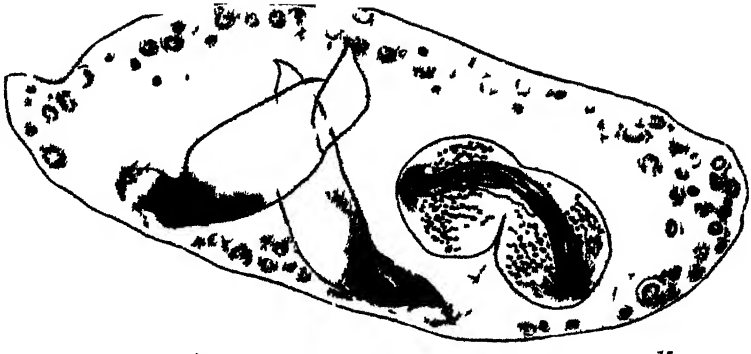
*Oxymonas grandis*

Fig 15 Telophase nucleus in posterior part of body but rostyles have migrated anteriorly nuclear matrix distinct From W *dalbergiae* 940 Holl H

Fig 16 Telophase two daughter nuclei connected by cablelike portion of the spindle and accompanied by well developed rostyles (anterior and recurvent portions) typical blepharoplast and flagella arrangement From W *tectonae* 940 S H

Fig 17 Parasitized animal atypical nucleus crowded posteriorly by abundance of small rods, rostyle extraordinarily pale From W *tectonae* C H

Fig 18 Nuclear matrix, blepharoplast and flagella arrangement From W *dalbergiae* S H



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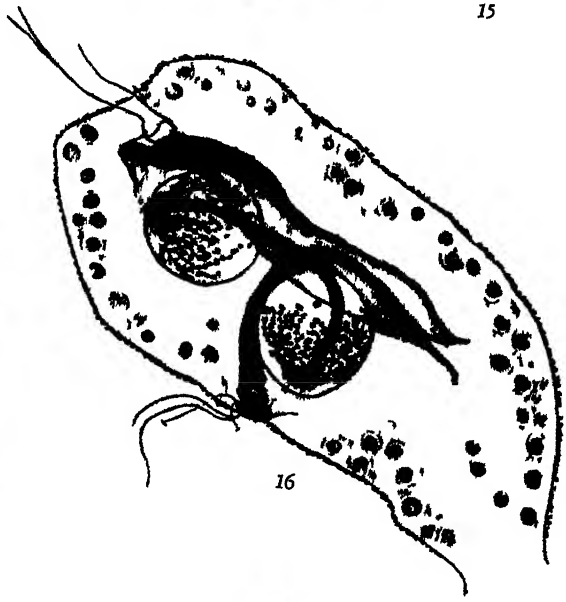

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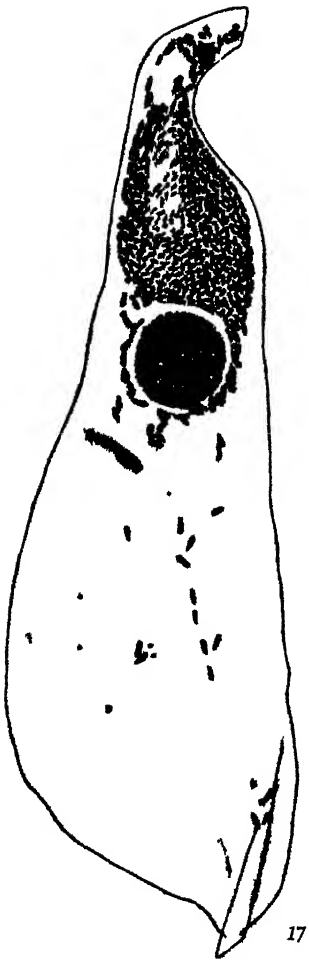

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## PLATE 6

*Oxymonas grandis*

Fig. 19. Holdfast attachment to the chitinous lining of the termite gut; anterior and recurvent portions of the axostyle. From *N. dalbergiae*. Sections. S-D-E.

Fig. 20. Plasmolysis: initiated by splitting of rostellum; old holdfast still functioning but new growth has replaced almost all of the older structures in the rostellum. From *N. dalbergiae*. S H.

Fig. 21. Early reorganization of nucleus carrying remnant of old cablelike portion of spindle. From *N. dalbergiae*. S-H.

Fig. 22. Post-telophase anterior migration of the nuclei. See fig. 21 for detail.

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## PLATE 7

*Oxymonas grandis*

Fig. 23. Reorganization period: arrowhead tip on axostyle, indicating immaturity. From *N. dalbergiae*. S-H-F.

Figs. 24 and 27. Telophase: binucleate preceding plasmotomy; no karyosome but numerous clumps of chromatin. From *N. tectonae*. S-H.

Fig. 25. Detail of nucleus of fig. 23; large karyosome present.

Fig. 26. Extraordinary looped growth of recurvent portion of axostyle fibers into cytoplasmic protuberance. From *N. dalbergiae*. Holl-H.

Fig. 27. See notation for fig. 24.

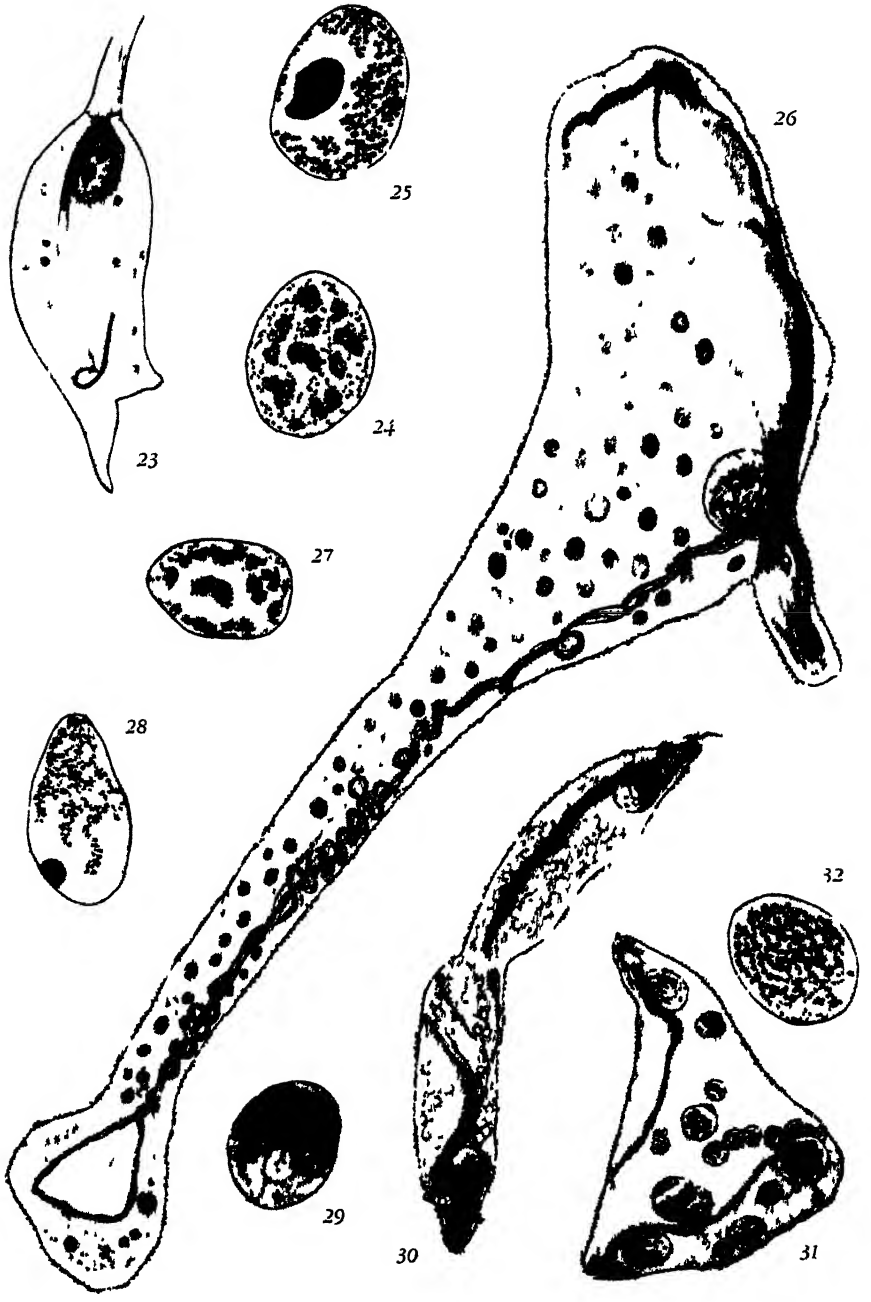
Fig. 28. Reorganization of nucleus; karyosome present. From *N. tectonae*. Section. Holl-H.

Fig. 29. Anterior nucleus of fig. 30.

Fig. 30. Late plasmotomy. Contrast this dwarf with the giant in fig. 26. From *N. dalbergiae*. S-H.

Fig. 31. Plasmotomy: earlier than fig. 30; many *Sphaerita*. From *N. dalbergiae*. S-H.

Fig. 32. Anterior nucleus in fig. 31.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## PLATE 8

Figs. 33, 34, 36, *Orymonas grandis*

Figs. 35, 37, 38, *Orymonas megakaryosoma*

Fig. 33. Post telophase anterior migration of nuclei; nuclear matrix; modified arrowhead formation of axostyles; dividing rostellum. From *N. dalbergiae*. S-H-F.

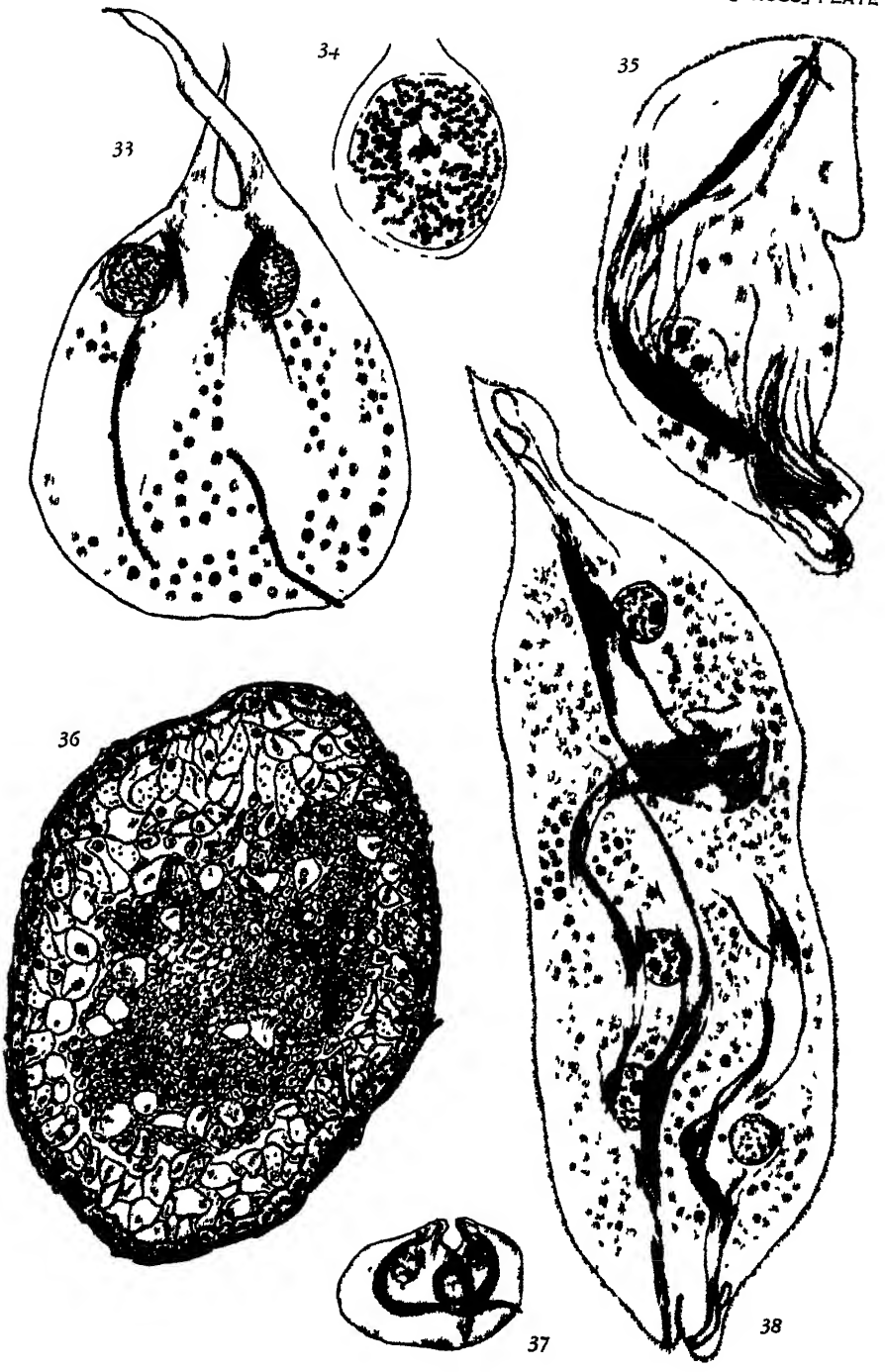
Fig. 34. Nucleus and matrix of fig. 33. Central splotch is probably remnant of earlier karyosome.

Fig. 35. Reorganization stage: new rostellum forming; old holdfast present. From *Glyptotermes* sp. nov. S H.

Fig. 36. Cross section of gut of *N. dalbergiae:* frequent and peculiar arrangement of protozoans. *Orymonas* may be recognized by its spherules, *Caduceia* by its much coiled parabasal, *Calonympha* by its sheaflike clustering of axostyles, *Devescovina* and *Foaina* by their small size and central position. × 63, but protozoans drawn larger for clarity. Fl-R.

Fig. 37. Early plasmotomy: dwarf, rostellum splitting and arrowhead tips on axostyles. S H.

Fig. 38. Plasmotomy: giant; four nucleate animal; contrast with figure 37. S H.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## PLATE 9

*Oxymonas megakaryosoma* sp. nov. from *Glyptotermes* sp. nov.

Fig. 39. Detail of axostyle shoulder, blepharoplast arrangement, and nucleus. Fl-R.

Fig. 40. Detail of axostyle shoulder and nuclear matrix. × 1540. S-H.

Fig. 41. Prophase: early spindle; chromatin massing at equator. S-H.

Fig. 42. Prophase: no karyosome, but clumps of dark-staining material which are possibly its remnants. S-H.

Fig. 43. Abnormally broad rostellum showing cytoplasmic fibers as recurvent portion of axostyle. S-H.

Fig. 44. Entire: for fig. 41; unusual anterior position for prophase nucleus.

Fig. 45. Prophase: earlier than fig. 41; karyosome in two clumps; nucleus in same position as in fig. 44. S-H.

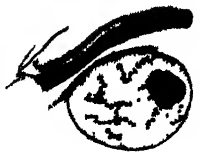
Fig. 46. Prophase nucleus: karyosome breaking up; two small granules with halo. Fl-R.

Fig. 47. Entire: usual position of posteriorly migrating prophase nucleus; axostyle largely degenerated. S-H.

Fig. 48. Prophase: nucleus of fig. 47, with delicate, amorphous nuclear matrix.

Fig. 49. Prophase: nucleus showing degeneration of dark-staining clumps to give discrete granules. S-H.

Fig. 50. Anaphase: cablelike portion of spindle; no karyosome; continuous matrix for nucleus and axostyle; young axostyle with anlagen of anterior and recurvent portions situated along the peripheral boundary of the nuclear matrix.



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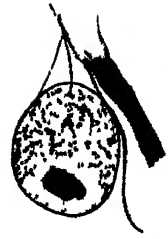

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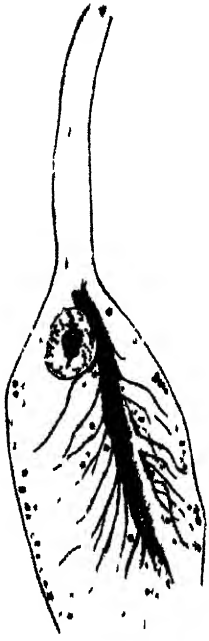

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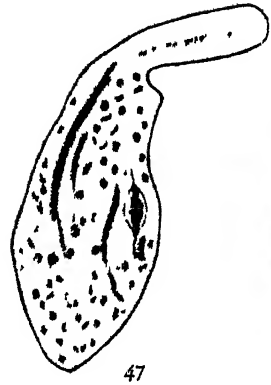

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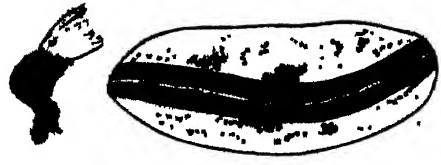

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## PLATE 10

*Oxymonas megakaryosma* sp. nov. from *Glyptotermes* sp. nov.

Fig. 51. Late anaphase: cablelike portion of spindle much arched; karyosome-like bodies with chromatin massed at either pole; axostyles along the peripheral boundary of the nuclear matrix. Compare with fig. 12. S-H.

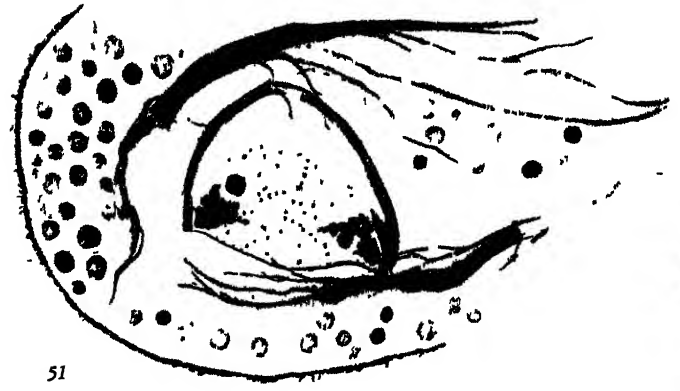
Fig. 52. Metaphase: chromatin massed at equator, forming a girdle; nuclear matrix distinct. Fl-R.

Fig. 53. Telophase: unequal division of the nuclear substance between the two daughters; distal polar view of the larger nucleus. S-H.

Fig. 54. Trinucleate animal with only two axostyles. S-H.

Fig. 55. Trinucleate animal: post-telophase anterior migration of nuclei preceding plasmotomy; no cytoplasmic spherules. S-H.

Fig. 56. Telophase: fibrillar nuclear matrix with posterior nucleus in addition to amorphous matrix surrounding both nuclei. Fl-R.



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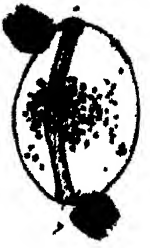

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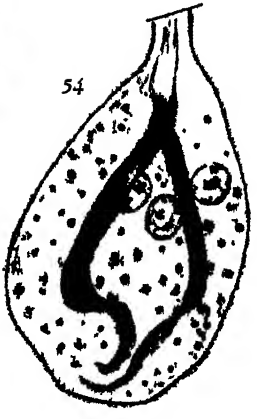

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## PLATE 11

*Oxymonas notabilis* sp. nov. from *Neotermes howa*

Fig. 57. Prophase: nucleus and accompanying clear area with fibrous boundary; probably an elongation of amorphous nuclear matrix. Z-R.

Fig. 58. Prophase: very early spindle; distinct nuclear matrix. S-H.

Fig. 59. Entire: prophase; see fig. 57.

Fig. 60. Prophase: tubular achromatic figure; chromatin massed at equator; achromatic figure occupies only a small fraction of nuclear volume. Compare with fig. 66. S-H.

Fig. 61. Prophase: later than fig. 60; karyosome present; cablelike portion of spindle arched. Z-R.

Fig. 62. Entire: nucleus in usual late prophase position. Fig. 61 gives detail.

Fig. 63. Early anaphase: matrix continuous for nucleus and axostyle; axostyles attached to poles of intranuclear spindle by fibrils. Fl-R.

Fig. 64. Entire: usual anaphase position for nucleus; orientation parallels long axis of body; fig. 63 gives detail.

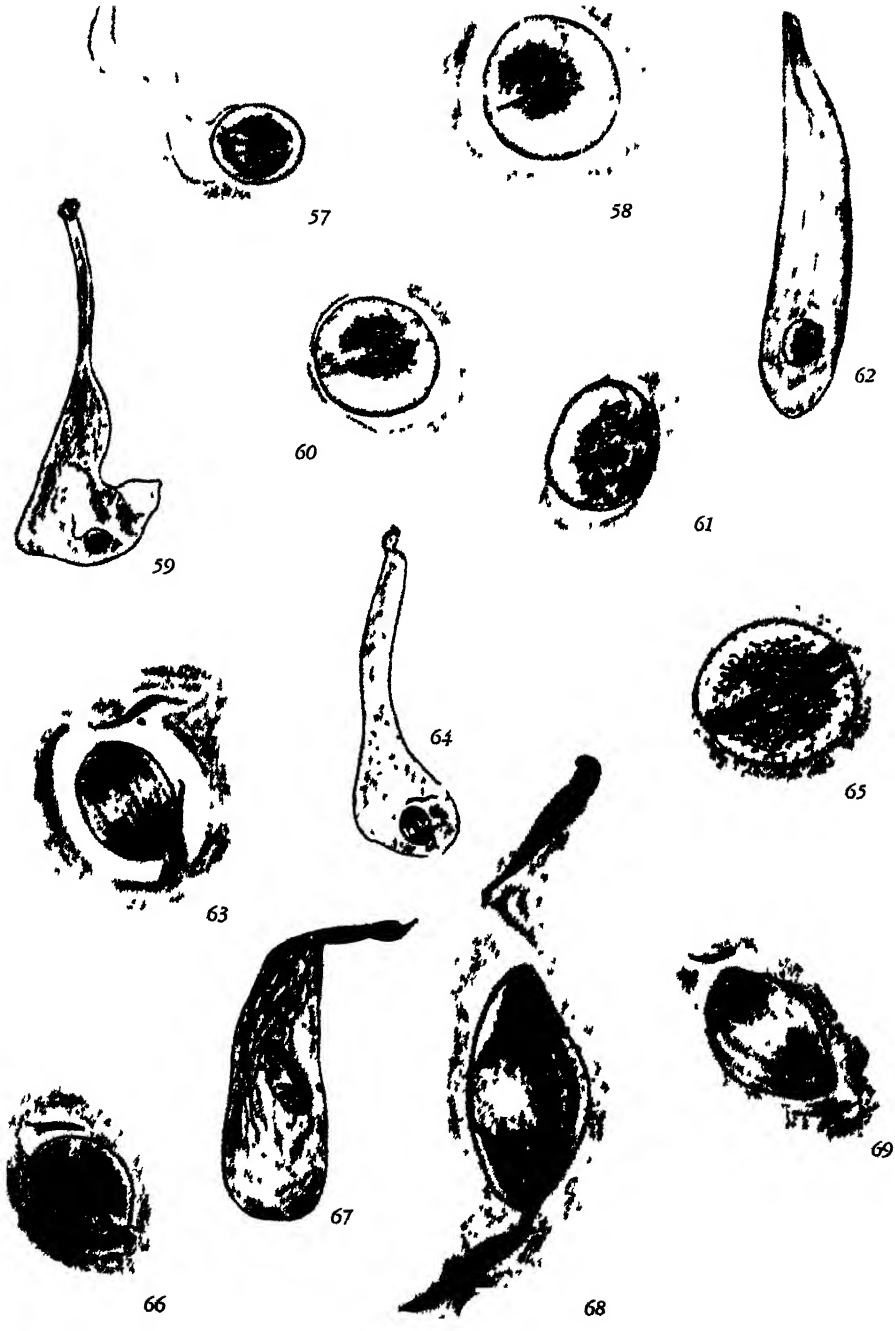
Fig. 65. Late prophase: cablelike portion of intranuclear spindle. S-H.

Fig. 66. Metaphase: cablelike portion of spindle much arched; typical, massed chromatin girdle; definite anlagen of axostyles; distinct nuclear matrix. Compare with figs. 58 and 60. S-H.

Fig. 67. Entire: anaphase nucleus located more anteriorly than usual. S-H.

Fig. 68. Anaphase: detail of nucleus in fig. 67.

Fig. 69. Anaphase: an intermediate stage between the nuclei of figs. 66 and 68. Z-R.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## PLATE 12

*Oxymonas notabilis* sp. nov. from *Neotermes hova*

Fig. 70. Entire: early telophase; polar axis of nucleus perpendicular to long axis of the body. S H.

Fig. 71. Telophase: fibril joining axostyle to anterior pole of intranuclear spindle. S-H.

Fig. 72. Telophase: cablelike portion of spindle showing torsion similar to fig. 13, of *O. grandis*. S-H.

Fig. 73. Early telophase: detail of nucleus of fig. 70; torsion of cablelike portion of spindle.

Fig. 74. Entire: binucleate, both nuclei in anaphase stage; atypical position for anaphase. S-H.

Fig. 75. Detail for fig. 74: two axostyles (half the usual number and more mature). Compare with fig. 63.

Fig. 76. Entire: usual position of the nucleus at this stage; see fig. 72 for detail.

Fig. 77. Metaphase: binucleate; young axostyles for one nucleus; typical position and orientation of nuclei. S-H.

Fig. 78. Entire: binucleate in anaphase stage; only half the usual number of axostyles. S-H.

Fig. 79. Entire: telophase, but nucleus not perpendicular to long axis of body; degenerating axostyle. S H.

Fig. 80. Detail of nucleus of fig. 79: telophase stage; daughter nuclei separated by considerable length of cablelike portion of spindle.

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## PLATE 13

*Oxymonas notabilis* sp. nov. from *Neotermes howa*

Fig. 81. Detail of left nucleus in fig. 85: cablelike portion of spindle present; anterior and recurvent portions of axostyle evident; definite matrices for nucleus and axostyle.

Fig. 82. Entire: post-telophase anterior migration of nuclei; cablelike portion of spindle joins nuclei; two blepharoplast granules barely detectable at axostyle shoulder. 940. S-H.

Fig. 83. Entire: post telophase anterior migration of nuclei; nuclei are more mature than in fig. 82, having lost their old spindle fibers and having developed karyosomes; old axostyle remarkably persistent. S-H.

Fig. 84. Plasmolysis: late. S H.

Fig. 85. Early plasmolysis, following post-telophase anterior migration of the nuclei. S-H.

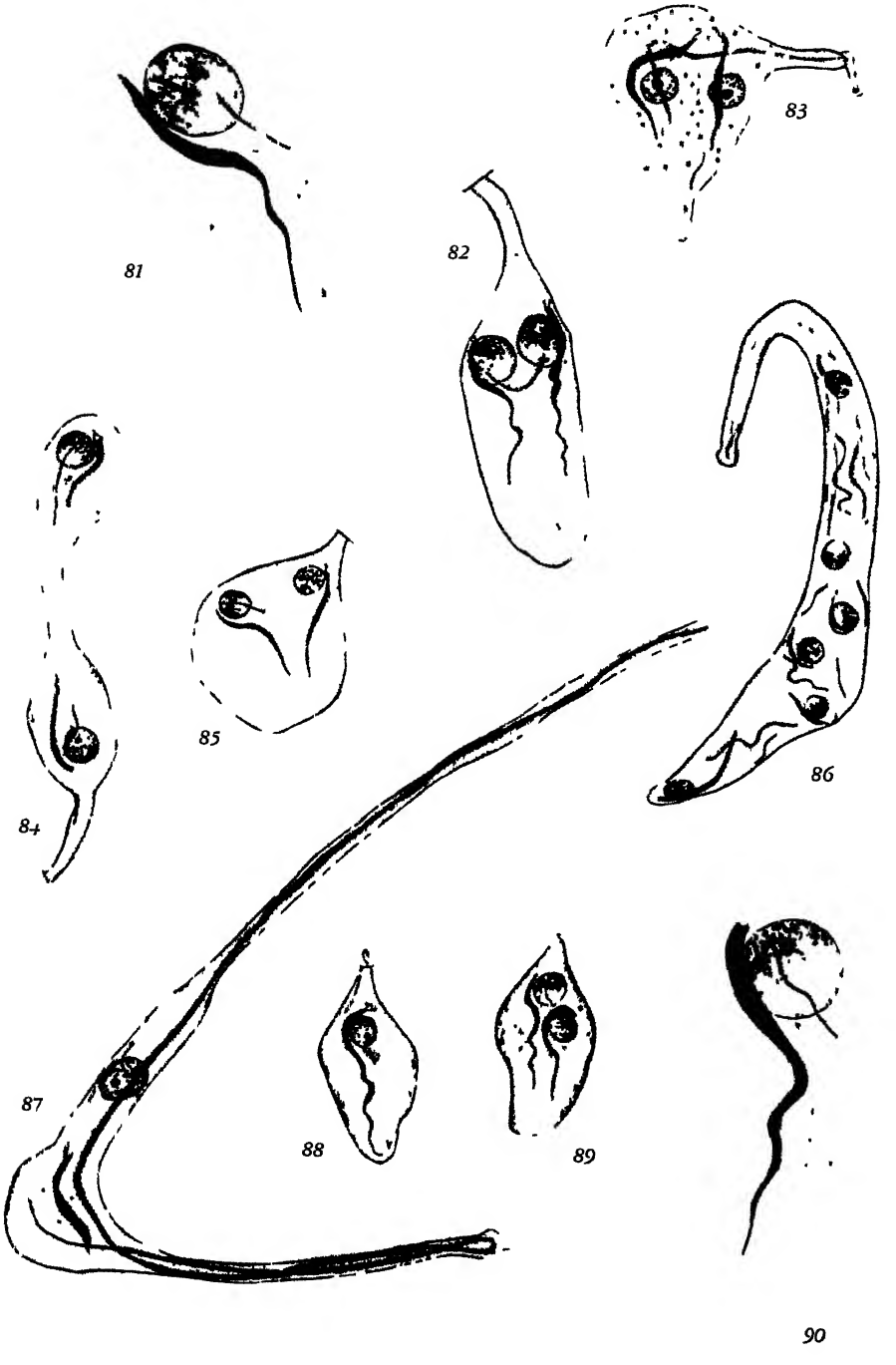
Fig. 86. Early plasmolysis of a 6-nucleate animal. Compare with fig. 31, *O. grandis*. Z R.

Fig. 87. Looping of recurvent portion of axostyle into cytoplasmic protuberance; retracted axostyle shoulder. S-H.

Fig. 88. Very young animal in which the axostyle matrix approaches the appearance of the "axostyle sleeve" described by Kofoid and Swezy. S-H.

Fig. 89. Post-telophase anterior migration of the nuclei. S-H.

Fig. 90. Detail of left nucleus of fig. 89: sharply defined matrix for axostyle; anterior and recurvent portions of axostyle.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## PLATE 14

*Barroella coronata* sp. nov. from *Neoterpes howa* var. *mauritiana*

Fig. 91. Entire: prophase nuclei in a somewhat anterior position; old axostyles resorbed. 940. Fl-H.

Fig. 92. Entire: giant; anaphase stage; old axostyles degenerating. Z H.

Fig. 93. Prophase: detail of fig. 91; definite nuclear matrix and spindle formation; achromatic figure occupying only a small part of nuclear volume. × 2280.

Fig. 94. Entire: metaphase; 25 nuclei in almost an identical kinetic stage; 1 nucleus in interkinetic condition; a few strands of old axostyle persisting. × 940. Z-H.

Fig. 95. Metaphase: chromatin massed to form equatorial girdle; achromatic figure occupying most of the nuclear volume; detail of nucleus from fig. 94. × 2280.

Fig. 96. Entire: 68 nuclei in telophase, 3 in anaphase; 123 axostyles; many telophase nuclei accompanied by the typical combination of flagella, blepharoplasts, and axostyle. S H.

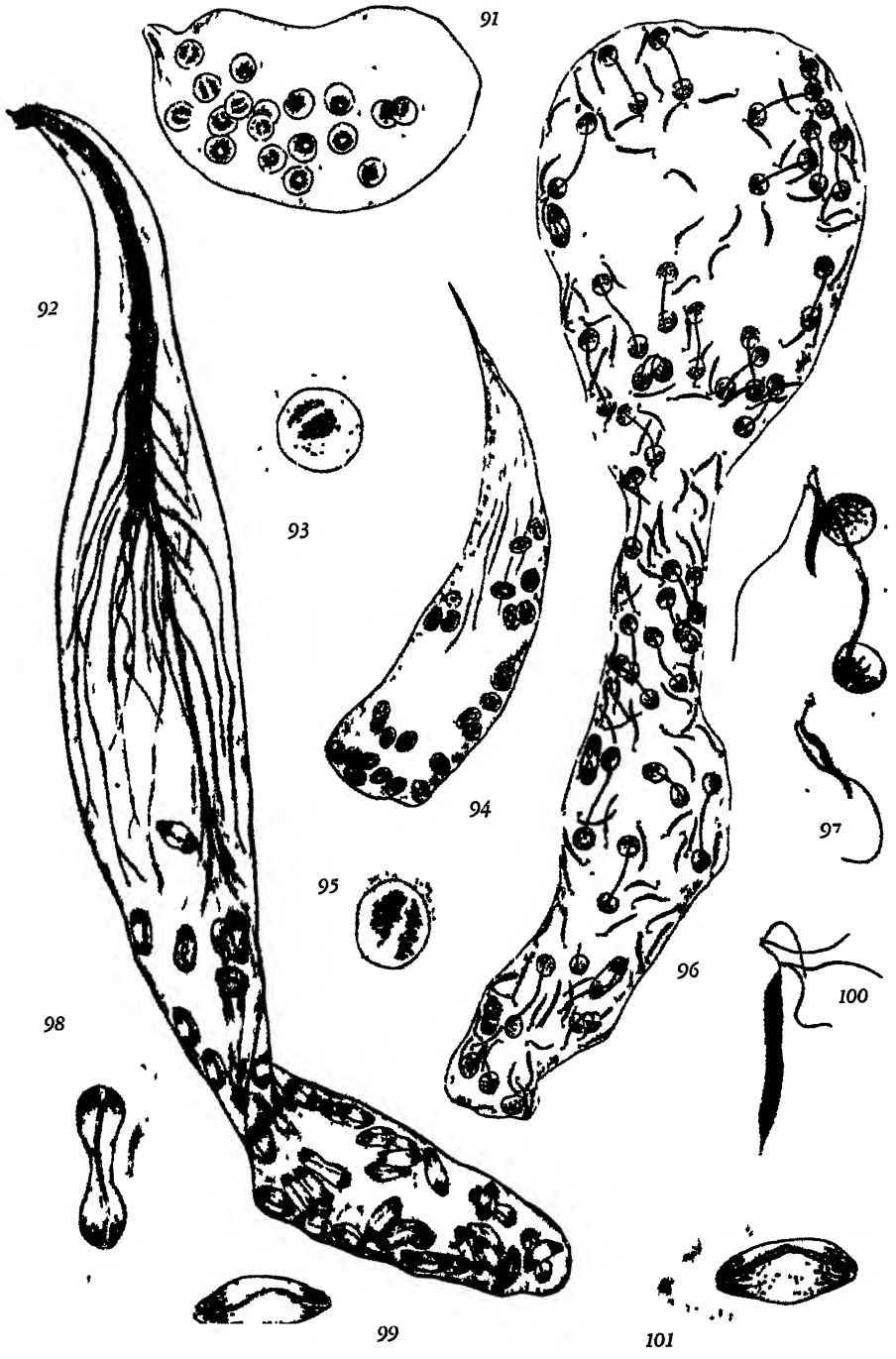
Fig. 97. Detail of telophase nuclei from fig. 96; some of the flagella have been lost.

Fig. 98. Detail of late anaphase nucleus in fig. 92.

Fig. 99. Detail of earlier anaphase nucleus in fig. 92.

Fig. 100. Detail of axostyle, blepharoplasts, and flagella arrangement in fig. 96.

Fig. 101. Anaphase nucleus in fig. 96: definite nuclear matrix; cablelike portion of spindle.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## PLATE 15

*Barroella coronaria* sp. nov. from *Neotermes howa* var. *mauritiana*

Fig. 102. Plasmolysis: axostyles dragging across cytoplasmic connection between body and "bud." S-H.

Fig. 103. Reorganization: "*Microrhopalodina*" stage, the final product of plasmolysis. S-H.

Fig. 104. Plasmolysis in small animal, producing probably two animals similar to fig. 103. S-H.

Fig. 105. Plasmolysis in larger animal, producing probably six animals similar to fig. 103. S-H.

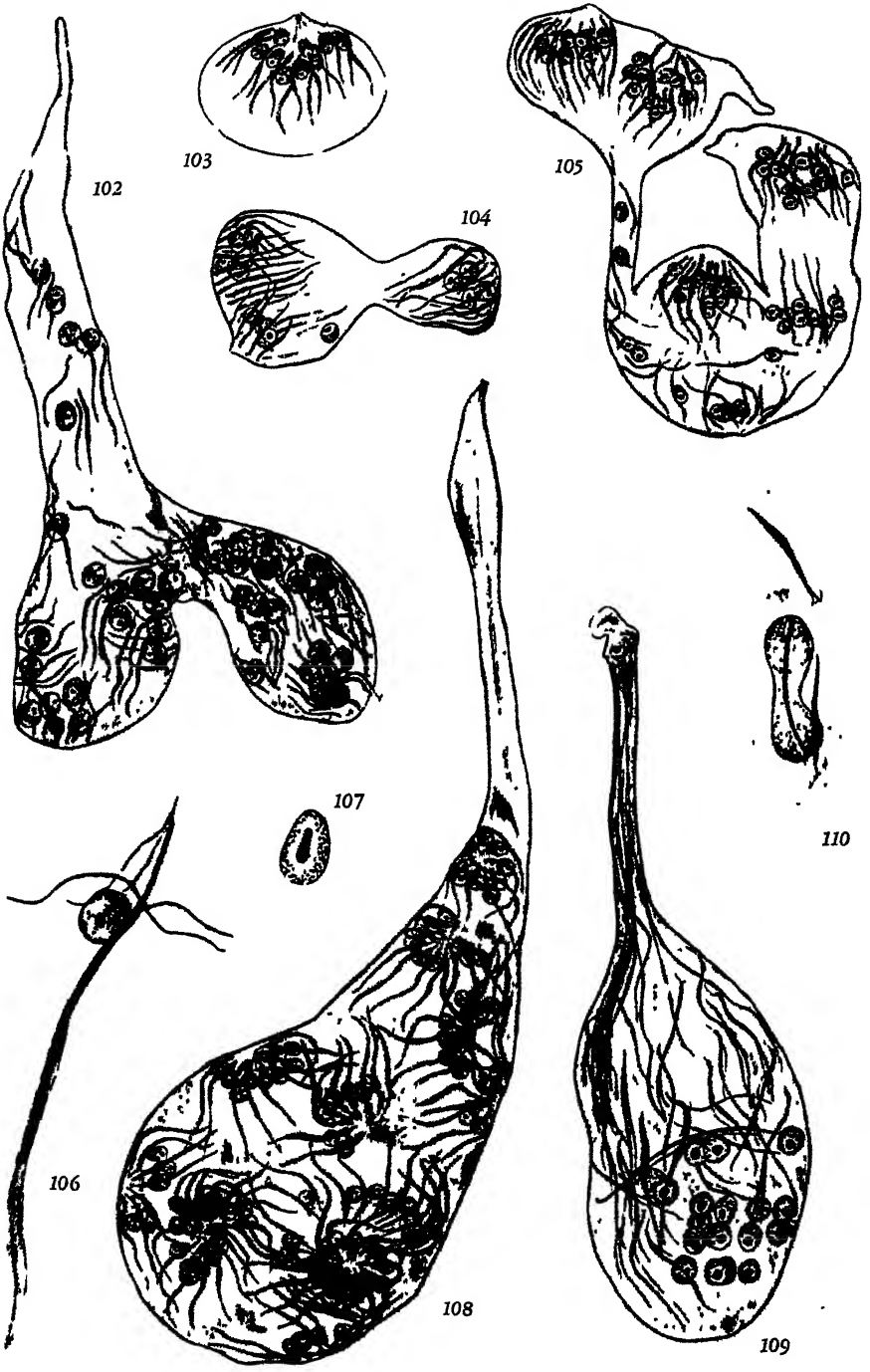
Fig. 106. Detail of nucleus, axostyle, blepharoplast, and flagella association from fig. 108.

Fig. 107. Nucleus from animal similar to fig. 103: extraordinary elongation of karyosome. S-H.

Fig. 108. Entire: modified post-telophase anterior migration of nuclei, resulting in the localization in whorls (or corona) of nuclei throughout the body. This is evidently only one stage earlier than fig. 105. Fl-H.

Fig. 109. Entire: modified post-telophase anterior migration of nuclei, indicated by the paralleling orientation of the young axostyles in an anterior direction; recurvent portion of the axostyle stained dark. S-H.

Fig. 110. Early telophase nucleus, showing the origin of the two axostyles along the boundary of the nuclear matrix and the surrounding cytoplasm. S-H.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## PLATE 16

*Barroella coronaria* sp. nov. from *Neotermes howa* var. *mauritiana*

Figs.111–116

Fig. 111. Detail of nucleus from fig. 112: accumulation of chromatin granules around the cablelike portion of the spindle, forming the karyosome.

Fig. 112. Animal containing 91 axostyles and 114 nuclei (counting as 8 the 4 incompletely divided nuclei). Almost all of the nuclei verify the condition shown in fig. 111. S H.

Fig. 113. Detail of nucleus and axostyle from fig. 115.

Fig. 114. Early metaphase: girdle not yet compacted; cablelike portion of spindle well developed; chromosomal fibers evident. S H.

Fig. 115. A modified post telophase anterior migration of nuclei; majority of axostyles oriented toward the anterior, and less mature than those seen in "corona" stage. S-H.

Fig. 116. Early anaphase. S-H.

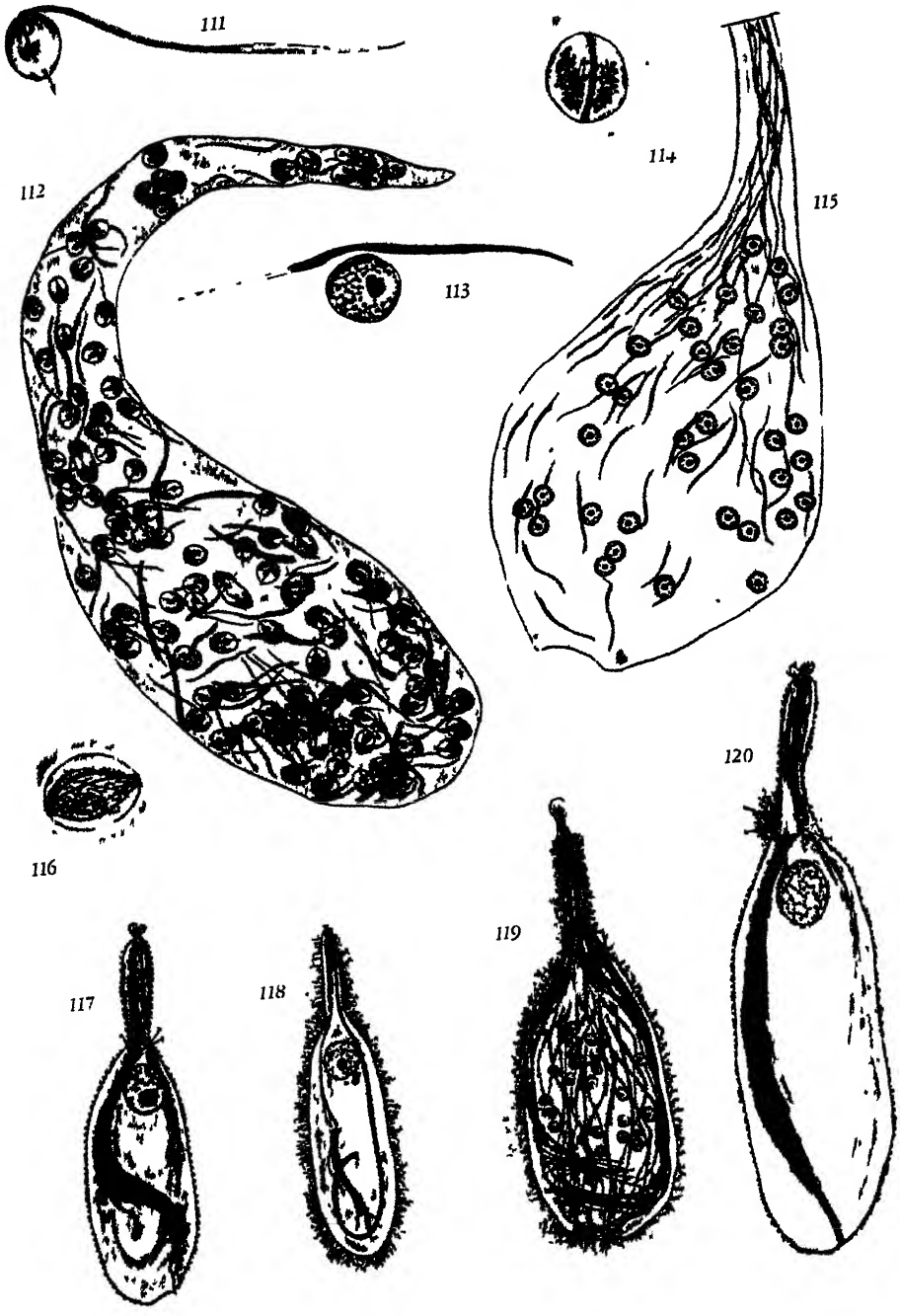
Diagnostic features and characteristic parasites of four Oxymonadinae, all drawn 550 × the measurement for the medians

Fig. 117. *Oxymonas megakaryosoma* sp. nov. from *Glyptotermes* sp. nov.

Fig. 118. *Oxymonas notabilis* sp. nov. from *Neotermes howa*.

Fig. 119. *Barroella coronaria* sp. nov. from *Neotermes howa* var. *mauritiana*.

Fig. 120. *Oxymonas grandis* Cleveland from *Neotermes dalbergiae* and *Neotermes tectonae*.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# GIGANTOMONAS HERCULEA DOGIEL
# A POLYMASTIGOTE FLAGELLATE WITH FLAGELLATED AND AMOEBOID PHASES OF DEVELOPMENT

BY

HAROLD KIRBY

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# GIGANTOMONAS HERCULEA DOGIEL A POLYMASTIGOTE FLAGELLATE WITH FLAGELLATED AND AMOEBOID PHASES OF DEVELOPMENT

BY

HAROLD KIRBY

## INTRODUCTION

THE PROTOZOÖLOGIST V. A. Dogiel, traveling in British East Africa in 1914, examined the termite *Hodotermes mossambicus* for intestinal protozoa. He found a number of flagellates and published accounts of them in 1916, 1917, and 1922. In 1934 and 1935 I examined many colonies of Hodotermitinae in Tanganyika Territory and various parts of South Africa. This report is an account of one of the flagellates, the only polymastigote that I observed. The other flagellates are hypermastigotes. In addition to the flagellates, there are sometimes present small amoebae and a species of *Nyctotherus*. Dogiel reported a small trichomonad, *Trichomonas macrostoma*, in all specimens of *Hodotermes mossambicus* that he examined; but I did not find that flagellate.

In his paper on the Tetramitidae (1916) Dogiel put different stages of the polymastigote flagellate, of which I am now giving an account, into two different genera. He named the flagellated, nondividing form *Gigantomonas herculea*, and the amoeboid and dividing forms *Myxomonas polymorpha*. A suggestion that *Myxomonas* and *Gigantomonas* are the same flagellate was published in a paper by Connell (1932). He thought it possible that the *Myxomonas* types represent only dividing and degenerating individuals of *Gigantomonas herculea*. My studies of living material in Africa convinced me that the amoeboid forms are not degenerating, but that they represent a phase in development that is as truly amoeboid as are comparable phases in *Dimastigamoeba gruberi* and *Tetramitus rostratus* (Bunting, 1926; Hollande, 1942). This phenomenon has not before been reported as such in a flagellate so complex as *Gigantomonas herculea*.

Two protozoa are known in which, as a normal event in the life cycle, there is persistence of the paradesmose in a binucleate, amoeboid phase: *Gigantomonas herculea* and *Dientamoeba fragilis*. These species differ greatly from one another in many respects, and the evolutionary relationship between the two seems to be remote. Nevertheless, the feature in which they are similar probably has significance in relation to the systematic position of *Dientamoeba*, as I have brought out in a section of this paper.

A fellowship from the John Simon Guggenheim Foundation made it possible for me to collect the specimens. Assistance in the work in Africa was given by Margaret Thomson Kirby. Technical assistance in preparation of the material and in a preliminary survey of the slides was given by Dr. Joy

Barnes Cross. Many of the drawings were made by Mr. Carl M. Stover, Miss Ruth Abbott, and Mrs. Marietta Voge. The Work Projects Administration made possible the translation of the Russian article by Dogiel, and grants from the Research Committee of the University of California furthered the work. The termites were determined by Professor A. E. Emerson.

## MATERIALS AND METHODS

The Hodotermitinae of Africa are differentiated into three well-marked types, which have been assigned to different genera (Emerson, 1942). In North Africa, *Anacanthotermes ochraceus* is present. In East Africa there is *Hodotermes mossambicus*. That species extends into South Africa, where *Microhodotermes viator* is also present. In both *Hodotermes* and *Microhodotermes* several other African species have been described, and subspecies have been differentiated, but Professor Emerson thinks that in Africa there may actually be only one species of each. The flagellates of *Anacanthotermes,* which correspond closely, if they are not identical, in several species of this genus of termites in Africa and Asia, are very different from those of *Hodotermes* and *Microhodotermes*. In its flagellate faunules, *Anacanthotermes* is markedly separated from the other two genera. *Hodotermes mossambicus* and *Microhodotermes viator* have flagellate faunules that are similar but not identical: some species of flagellates in both are the same, others closely related. My material, secured from many different localities in South Africa, probably contains a number of the types reported as species by Fuller (1921). Even if there is a valid differentiation of the termites, however, they all—within each genus—appear to be identical in protozoan faunules. *Gigantomonas herculea* occurs in both *Hodotermes mossambicus* and *Microhodotermes viator*.

Hodotermitinae live in a very different manner from Kalotermitidae. The latter usually occur in more or less sound dead wood, within which all forms live in the cavities they make; the termites feed on this wood, and no form except the winged adult ever normally occurs outside the wood. Hodotermitinae inhabit nests underground; from the nest cavity galleries extend to the surface of the ground, and the termites may penetrate the walls of houses built of mud bricks. The workers forage in the bright sunshine from the openings at the surface of the ground. They collect such objects as grass stems, fine twigs, and pine needles, which they take into the burrows; and they ingest fragments of this vegetable matter.

The termites were collected either on the surface of the ground or by spading off the small mound of loose earth that often covers the entrance of burrows, and that may contain some workers and soldiers. After collection, it was found necessary to make preparations with as little delay as possible. Kalotermitidae could be put in closed containers with wood or paper and would often live indefinitely; after they had been placed four days or so on filter paper, division stages of the flagellates frequently resulted. When hodotermitins were put in containers with dry grass and some moisture, the population of protozoa became much reduced within a day or so, and the termites lived only a few days.

The preparations were made on cover glasses, fixed in Schaudinn's or Flemming's fluid, and stored in alcohol until I returned to Berkeley, when they were stained in Heidenhain's iron-haematoxylin or Delafield's haematoxylin. In addition, intestines were removed entire and fixed without opening them; these were subsequently sectioned and stained in the same manner as the cover-glass films. Studies of living material were made in the field.

## DOGIEL'S ACCOUNT OF GIGANTOMONAS HERCULEA AND MYXOMONAS POLYMORPHA

(Fig. A, 1–9)

Dogiel's article (1916) is in Russian and English. The latter section gives the report in abbreviated form; it is not a translation, but it is more than an ordinary summary. The résumé of his account which I give here, in order to show how published knowledge of the flagellate stood for thirty years, is written from a translation of the Russian text.

The preparations studied by Dogiel consisted mostly of films made directly from the contents of the intestine of *Hodotermes mossambicus* and fixed in Schaudinn's fluid. In addition to making these, he emptied the contents of the digestive tract into Gilson's, Schaudinn's, or Flemming's fluid in test tubes, and made whole mounts and sections from the material that settled in the tubes. He did not report any observations on living material.

Dogiel considered that the flagellated form to which he gave the name *Gigantomonas herculea* (fig. A, 1) greatly resembles *Trichomonas*, but is much larger, reaching a length of 60–70$\mu$. At that time those Trichomonadinae of termites that reach even larger dimensions—*Trichomonas termopsidis*, *Trichomonas immsi*, and *Pseudotrypanosoma giganteum*—were unknown. He believed that the structures of *Gigantomonas* and *Trichomonas* are altogether comparable to one another, though that is not true, as I will bring out later in my own account. He found four free flagella, all of which as shown in his figures are extended anteriorly; apparently, then, he compared these to the four anterior flagella of the trichomonad which he placed in the same paper in the subgenus *Tetratrichomonas*. These flagella were differentiated into one main flagellum, which is considerably longer than the whole body, and three very short accessory ones. He remarked upon the difficulty of seeing the flagella in his preparations, and it is clear that he did not observe them as they actually are.

He reported an undulating membrane which extends the full length of the body and is turned in a spiral. He described this membrane as not projecting above the surface of the body, but lying within a furrow, and thickening from its outer margin to its base. He designated the outer boundary as the marginal flagellum, which had no free terminal part. His observation of this structure led him to doubt the validity in *Trichomonas* of the differentiation of an independent chromatic basis (now known as costa), because in *Gigantomonas* he saw only a gradual thickening and intensification of staining, instead of a sharply differentiated structure. As I will show later, the structure which

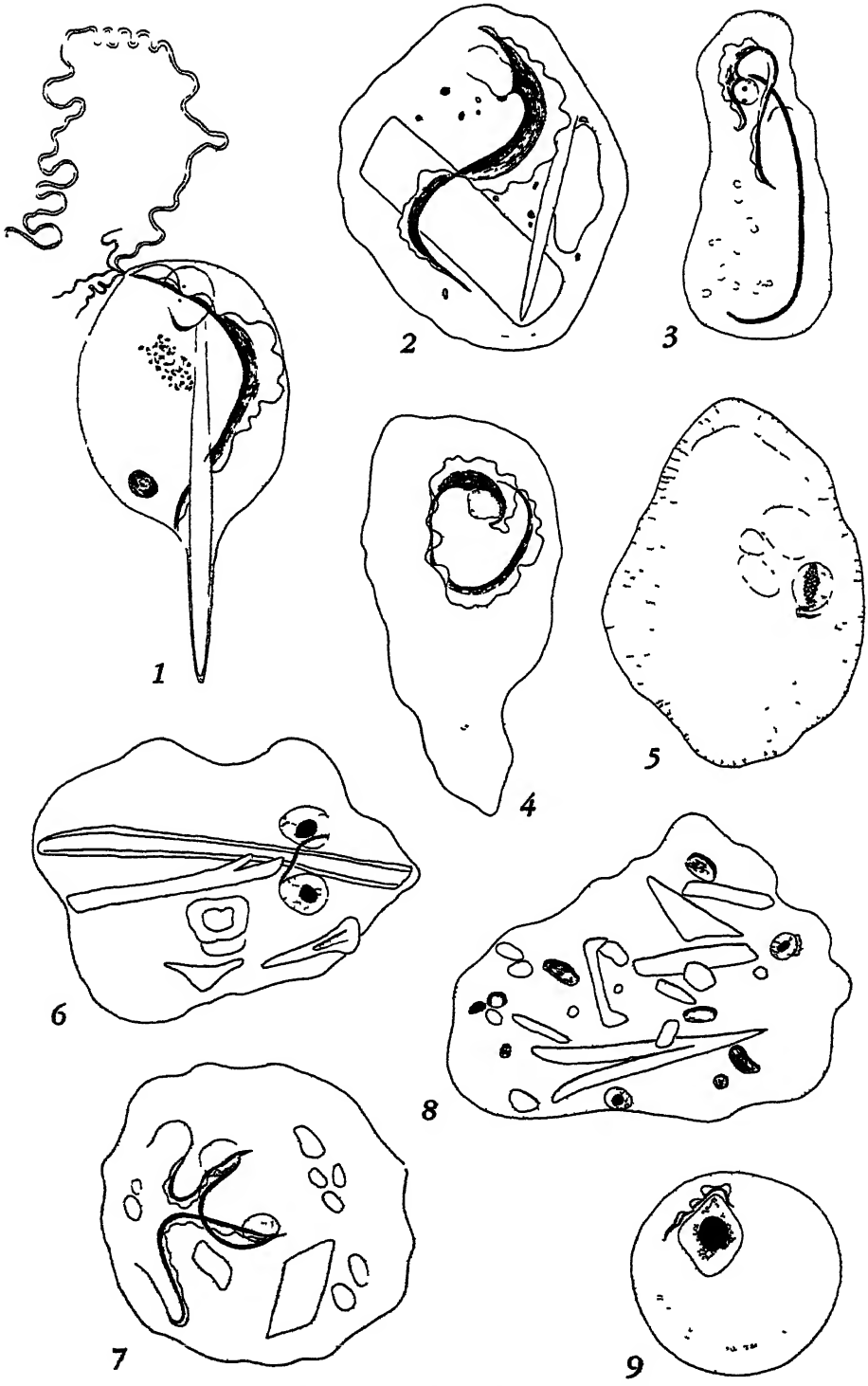


Fig. A. (For explanation of figure, see bottom of facing page.)

he regarded as the undulating membrane is the cresta, and the long free flagellum is the trailing flagellum, which under normal conditions follows the edge of the cresta.

He observed the axostyle as a thick spindle-shaped structure, projecting for a distance of 20–30$\mu$ from the main body and covered in the projecting part by a thin plasmatic film. He believed it to be composed of numerous fine fibrils, and stated that it does not stain with Heidenhain's iron-haematoxylin. In one specimen the axostyle was found entirely enclosed in an amoeboid body, and there were no flagella (fig. A, 2).

No division stages of the flagellated form named *Gigantomonas herculea* were found. Dogiel did not realize that he was observing these division stages in the amoeboid forms that he put into a separate systematic category as *Myxomonas polymorpha*.

Dogiel stated that in *Myxomonas polymorpha* the amoeboid state, which is infrequent in *Gigantomonas*, has become constant, and the cytostome and free flagella are lost. He described a series of forms, designated as types A to F. These forms were not supposed to be differentiated genetically; he was merely separating different phases of the life history.

Type A of *M. polymorpha* (fig. A, 3) is the form which, according to Dogiel, resembles *Trichomonas* most closely. Even more appropriately, he might have compared it directly with *G. herculea*, especially with the amoeboid, aflagellate specimen (fig. A, 2) which he had assigned to that species. Its size is comparable to that of *G. herculea*, and its shape, though "amoeboid" in outline, is somewhat similar. The organelles of type A consist of a single nucleus, an undulating membrane, and an axostyle. The nucleus, by Dogiel's account, is at one end of the body, is large and spherical, and contains a linin network and chromatin concentrated in one large karyosome or two to five smaller ones. With the nucleus is closely associated the anterior part of the undulating membrane which, he stated, resembles the membrane of *Gigantomonas*. He found a distinction between the two membranes in the structure of the exterior edge. In *Myxomonas* there are said to be more undulations and a supplementary filament parallels the border filament. Dogiel compared these two filaments to the two filaments that had been described in the membrane of some trichomonads; he considered one of them to be a border flagellum, which in some circumstances becomes separated. He failed to make the correct interpretation of the relationship between this structure and the flagellar organiza-

Fig. A. *Gigantomonas herculea* Dogiel from *Hodotermes mossambicus*. Diagrams copied from figures by Dogiel, 1916, labeled according to his designation. 1. *Gigantomonas herculea*. The supposed one long and three short anterior flagella are the trailing flagellum that follows the edge of the cresta and the proximal parts of the three true anterior flagella, the full length of which was not seen. 2. Amoeboid form of *G. herculea*, with the axostyle, large cresta, and ingested particles. 3–9. *Myxomonas polymorpha*. 3. Type A. This is much like fig. 2, except that the core of the axostyle is deep-stained. 4. Type B. Similar to A except that no axostyle is shown. 5. Type C. Large amoeboid body, one nucleus, small cresta, which Dogiel called an undulating membrane. 6. Type D. Early division with spindle, no axostyle or cresta. 7. Type D. Later stage, with well-developed crestas. 8. Type E. Large amoeboid body. Two nuclei, each associated with a small cresta; no spindle. 9. Type F. Small body, with one nucleus and small cresta, supposed to originate from type E. These diagrams vary in the scale of magnification.

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tion he had found in *G. herculea,* which I will bring out later. The axostyle was reported to consist of a lighter peripheral layer and a black axis (after Heidenhain's iron-haematoxylin). It was always found entirely enclosed in the body. Dogiel stated that there are no anterior flagella, but he was not certain on this point. He reported his observation of two specimens, each of which had two black granules at the anterior end of the membrane, and in one of these two fine threads extended from a granule out of the body (his pl. 1, fig. 13).

Dogiel described as type B of *M. polymorpha* (fig. A, 4) a form similar to type A but lacking the axostyle. In size type B is similar to the other form, but more small specimens occur. The membrane is more often coiled around the nucleus in one plane, as appears in the figure. The nucleus is said to lack a karyosome. Usually, as in type A, there are no food inclusions.

Individuals of type C (fig. A, 5) are reported to vary greatly in size and shape. They have the appearance of large amoebae. There is a single nucleus and a very small undulating membrane; axostyle and flagella are absent. The form is further distinguished from the preceding ones in the structure of the protoplasm. There is an outer zone of ectoplasm, which may vary in thickness in different parts of the body, and appears radially striated. The nucleus possesses a large spherical or more often elongated karyosome. Dogiel regarded the undulating membrane as rudimentary in character. A second filament, paralleling the border filament, can be found in some preparations. Usually there are no food inclusions. Some specimens of this type have become rounded up, and the cytoplasm is filled with chromidia that originate from the nucleus. He believed that these are forms preparing for encystment, and that their development is comparable to that of encysting phases of some "Trichonymphidae" in the same *Hodotermes.* He did not, however, find definite cysts with a cyst membrane.

Type D consisted of the forms showing various stages of nuclear division. According to his account, a fibrillar, stainable spindle develops on the surface of the nucleus; thread-formed chromosomes appear within the nucleus; and after nuclear division the separated nuclei continue to be connected by the spindle (fig. A, 6, 7), which elongates and sometimes becomes arched. In what he regarded as the early stages following this division (fig. A, 6), he observed no axostyle or undulating membrane. After the nuclei have returned to the resting condition, he stated, new undulating membranes begin to form. Dogiel found a series in growth of the membrane from a short filament closely applied to the nucleus to a structure as large as that in types A and B (fig. A, 7). Fission of the body separates the two nuclei with the corresponding undulating membranes, and divides the spindle into two parts. Dogiel believed that the spindle gives rise to the axostyle of the type A flagellates, but that in some forms it is absorbed so that type B flagellates without an axostyle result. Type D specimens almost always contained many food inclusions, consisting of particles of wood and other vegetable matter. Numerous spores, probably of fungi, were often also present.

Type E consisted of large amoeboid specimens with two nuclei (fig. A, 8).

With each nucleus is associated a very small undulating membrane, and no persisting spindle is present. Dogiel found small rounded bodies (fig. A, 9) with nuclei and small membranes like those in type E, and believed that these, type F, resulted from the breaking up of type E individuals into balls of cytoplasm, some of which might contain nuclei and others not.

Dogiel believed that type C, with a large amoeboid body, a single nucleus, and a small undulating membrane, represents the fundamental form of *Myxomonas polymorpha*. These individuals may undergo nuclear division, and become binucleate amoebae of type E, in which two nuclei and membranes of the same sort as in type C are present; but he did not report division stages leading to that form. He stated that these binucleate amoeboid forms divide, becoming smaller and giving origin to type D, or under abnormal conditions they break up into balls, giving type F. He found a division series in type D, and the result of division is an individual of type A or type B. Dogiel thought that the last two types might be concerned in sexual reproduction, but he had no evidence for that opinion.

My observations on the same flagellates, although not complete, have verified most of the descriptions that Dogiel gave, but since I have had more stages and have been able to work out more detail, a more plausible interpretation is now possible. That this is needed is obvious, in view of the way in which this remarkable flagellate has been misunderstood by protozoölogists.

## OBSERVATIONS ON LIVING MATERIAL

(Fig. B, 1–12)

Upon examination of the gut contents of *Hodotermes mossambicus* near Mbeya in Tanganyika Territory I found, in addition to the hypermastigotes and a small amoeba, flagellated polymastigotes corresponding to *Gigantomonas herculea* and rather large amoeboid forms corresponding to what Dogiel described as *Myxomonas polymorpha*.

The flagellated form, when unaltered, has a shape similar to that of many devescovinid flagellates. The body is elongate-oval in outline (fig. B, 1) and is circular in cross section. The axostyle is a refractile rod which extends beyond the posterior end of the body in a pointed spike. The cresta extends from the anterior end to near the posterior end, and it is turned in a spiral of about one gyre. It lies entirely within the cytoplasm, and it does not undulate; only the flagellum at the outermost edge undulates. At the anterior end is a mobile papilla along which the anterior flagella extend.

The body changes shape readily and generally at once assumes an amoeboid form when placed in 0.67 per cent salt solution. When a preparation is first made, one sees what appear to be small amoebae, many of which show a crenulated margin. After a few minutes the anterior flagella may appear and the organisms resolve themselves into typical flagellated *Gigantomonas herculea* as described in the preceding paragraph. As the preparation gets older they revert to amoeboid forms. Close observation of the amoeboid forms reveals the clear area of the cresta, which is curved more or less in a circle. In many specimens there is no evident movement; in others a feeble undulation

of the trailing flagella within the cytoplasm or on the surface may be observed. The axostyle may be completely enclosed within the cytosome. These amoeboid forms of *Gigantomonas* (fig. B, 2, 3), which evidently are the result of exposure to an unfavorable environment, correspond to what Dogiel described as Type A of *Myxomonas polymorpha*. They are the forms that are usually to be found in preparations, since alteration of form occurs rapidly. The anterior

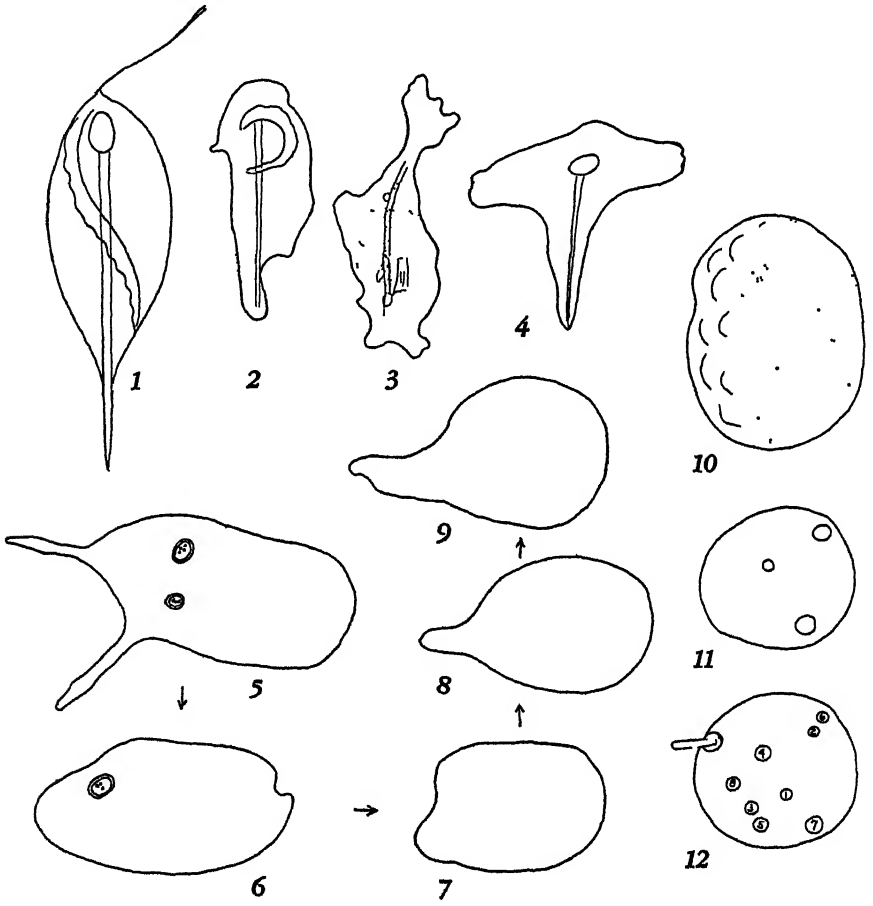


Fig. B. *Gigantomonas herculea* from *Hodotermes mossambicus*. Freehand sketches made from living material, not drawn at the same relative magnification. Fig. 1 is on a larger scale than the others. 1. Flagellated form with posteriorly projecting axostyle. 2. Cresta curved in anterior part of body, which has an amoeboid form. 3. Clear ectoplasm spread out in a thin layer against the cover glass. 4. Amoeboid form, which showed activity in drawing in parts of the body and changing shape. This eventually drew up into a rounded form, in which the axostyle trunk was curved and the cresta could be seen. 5–9. Forms assumed at intervals of a few minutes by the same amoeboid *Gigantomonas*. In 5 and 6 vacuoles are shown which contained a small, active flagellate. 10. A large amoeboid form with a broad, clear protoplasmic zone showing many pseudopodial protuberances. This broad zone appeared on one side of many apparently normal specimens. 11. Amoeboid body containing three collapsing vacuoles, only one of which was evident at a time. 12. Amoeboid body containing eight collapsing vacuoles. Only one vacuole was seen to collapse at once, and there was generally a rather long interval before another one collapsed. Vacuole 4, however, remained expanded while three others formed and collapsed; eventually vacuole 4 collapsed also. At one edge an inclusion is being extruded.

flagella are sometimes seen and sometimes not; that may be a consequence of the technique of preparation, or perhaps the flagella are cast off.

The larger amoeboid forms (fig. B, 10) suggested the amoebae which I had observed a short time before in *Cubitermes* sp. near Nairobi, and which have been described by Henderson (1941). There were some amoeboid forms which in size and activity resembled *Endamoeba pellucida,* and others which suggested the larger amoebae of *Cubitermes—E. granosa* and *E. lutea*—in size, and were very sluggish in activity. As it was not possible to study in living material the state of nuclear development in these amoeboid forms, they cannot be fully correlated with the stained preparations, but there is no doubt that they belong in the life history of *Gigantomonas herculea.* The true amoebae found in the preparations were very small forms, not abundant, and present in only a few of the termites. They were very sluggish, with a *"limax* type" of locomotion.

Some of the smaller amoeboid forms showed in the cytoplasm slow streaming in various directions, much slower than is usual in amoebae of the *proteus* type. Most of the larger amoeboid forms remained rounded up in the salt solution in which they were examined. Others were observed to change shape slowly. Long narrow processes now and then appeared; often these seemed to be drawn out in locomotion, with their ends attached to the glass. Eventually some such processes snapped and the parts belonging to the cytosome were drawn in. Broad pseudopodia were slowly pushed out. One or more lobes were thrust out from various parts of the body; these gradually changed their outline and position, or were withdrawn (fig. B, 5–9). Often the changes were so slow that they could scarcely be watched, and practically no internal streaming could be seen. Some of the organisms showed a *limax* type of locomotion, in limited degree, but this was not frequent or continuous enough to be altogether comparable to that of *Endamoeba pellucida.*

In some of the amoeboid forms practically no ingested bodies were seen; others contained a number of long vegetable fibers and fragments of plant material. From one specimen a fiber was observed to be extruded. Small hypermastigotes were enclosed within vacuoles in one specimen. Some contained a *Sphaerita*-like organism, and others had numerous larger structures in the cytoplasm, which evidently were the symbiote described below under the designation Ghl.

In some specimens a clear zone of ectoplasm was conspicuous; in others, except in the region of forming pseudopodia, there was no part free from minute, refractile cytoplasmic granules. Often a clear area was especially prominent on one side, knobbed by short amoeboid processes (fig. B, 10). Relatively clear cytoplasm was sometimes spread out against the glass, and granules in it were often arranged so as to form striations (cf. fig. A, 5).

The peripheral cytoplasm of some specimens contained many vacuoles, and some of these were observed to enlarge and collapse. In one specimen (fig. B, 12) eight vacuoles were seen to do this. Only one collapsed at a time, and there was a rather long interval before another did so. Apparently the same vacuole did not form again, since each one that collapsed appeared in a different part

of the body. One of those which eventually disappeared remained expanded while three others formed and collapsed. These collapsing vacuoles are probably not homologous with true contractile vacuoles; they are not constant in occurrence or number.

## STRUCTURE AND LIFE HISTORY

### The Flagellated Nondividing Form

(Pl. 17, fig. 1; pl. 18, figs. 2–6; pl. 19, figs. 7–8; pl. 20, fig. 11; fig. E, 1, 2)

On slide preparations very few flagellated specimens of *Gigantomonas herculea* are found in which the shape of the body is normal, because of the rapid alteration described above (p. 169). This is in marked contrast to the flagellates of the subfamily Devescovininae, and also to the hypermastigotes in the same material from *Hodotermes mossambicus* and *Microhodotermes viator*. The hypermastigotes on the same slides were in excellent condition, and served as an indicator of satisfactory technical procedure. *G. herculea* is more liable than most protozoa to changes in body form in the process of preparation of films, even though that is done as rapidly as possible. Dogiel's method, which I did not use, of opening the gut and dropping the contents directly into fixative may have preserved the body form better.

The two specimens of *G. herculea* represented by figure 1, plate 17 and figure 5, plate 18, in which the body shape seems not to have been much altered, have lengths of 59 and 72$\mu$ and widths of 36 and 44$\mu$. This agrees fairly well with Dogiel's dimensions of 60–70$\mu \times$ 30–35$\mu$. In specimens with altered body form the dimensions of the cytosome are not very significant. The axostyle, cresta, and nucleus, however, can be measured accurately, even in such specimens. In fifteen specimens the ellipsoidal nucleus had a length of 7 to 12$\mu$, a width of 5 to 8$\mu$. In ten specimens the anteromedial edge of the cresta had a length of 13 to 18$\mu$, the posteromedial edge 49 to 60$\mu$, and the external edge 80 to 94$\mu$. The length of trunk of the axostyle, from the posterior end of the nucleus, ranged from 53 to 71$\mu$.

At the place of origin of the anterior flagella a small protuberance is often seen (pl. 17, fig. 1), but it is not so prominently developed as the papilla usually is in devescovinids. There are three anterior flagella, which are fine and long. In six specimens in which they were seen distinctly they ranged in length from 50 to 95$\mu$. Dogiel saw the three slender anterior flagella, but he did not observe their full length. He stated that he had observed them only in fixed animals where they were greatly twisted and curled up, so he gave no measurements.

The trailing flagellum originates near the anterior end and runs posteriorly at the surface of the body, usually more or less parallel to the peripheral edge of the cresta (pl. 17, fig. 1; pl. 18, fig. 3). It does not, however, appear to be consistently attached to that edge of the cresta; its contact with the cresta is transitory. When the position of the cresta is shifted and it is turned around the anterior end (pl. 18, fig. 5), the trailing flagellum goes with it, so there is obviously a close relationship; and in living material the flagellum is on the surface of the body along the edge of the cresta. In most of the fixed material

the contact is broken, and as in the Devescovininae the separation takes place much more readily than it usually does in the undulating membrane of *Trichomonas*. Separation was complete in Dogiel's material, so much so that he considered this flagellum to be the main anterior flagellum, which was much stouter and longer than the others. The length of the trailing flagellum is greater than that of the body, so that it continues for a short distance beyond the posterior end of the cresta. It is cordlike, not very stout, but yet stouter than an anterior flagellum.

At the anterior end, near the origin of the flagella, there are two rather large deep-staining granules, generally unequal in size (fig. E, 1, 2). Occasionally, with a greater degree of destaining, the larger granule appears as a ring. The flagella do not originate directly from either of these granules, but from a small granule or pair of granules close to them. Sometimes there are clearly two separate small granules from which the anterior flagella originate in two roots, one of which divides into two flagella (pl. 18, fig. 3).

A sigmoid filament that meets the nuclear membrane is shown in figure 3, plate 19, and figure E, 1. It appears to meet the large granule, but probably it passes around it to meet one of the blepharoplasts. This nuclear rhizoplast is comparable to the one that I have found in various devescovinins. It appears most clearly in *Macrotrichomonas* (Kirby, 1942, pl. 16), and in that devescovinin, too, it has a similar relation to a large granule posterior to the ones in which the organelles originate.

The cresta is a prominent structure in the flagellated forms of *Gigantomonas herculea* (pl. 17, fig. 1; pl. 18, fig. 2). It is comparable in development to the crestas of species of *Macrotrichomonas*. As in flagellates of that genus, it may extend in an open, longitudinally directed spiral along the body, or may be turned transversely around the nucleus (pl. 18, fig. 5; cf. *Macrotrichomonas lighti,* Kirby, 1942, pl. 22). Its anteromedial edge is short, and is applied closely to the nuclear membrane (pl. 18, fig. 6). The cresta is entirely within the cytosome, like the crestas of Devescovininae; there is no reason for believing that it lies in a furrow of the body wall, as Dogiel supposed. Usually there is a clear space in the cytoplasm along one side and the inner edge. The cresta is rather markedly differentiated into two parts, like that of *Macrotrichomonas pulchra*. The inner part of the cresta, constituting more than half of its breadth, is flat and deep-staining. This part is bordered peripherally by a diffuse but well-defined margin, and beyond it is the clear outer part of the structure. This part is considerably longer at its margin than at its inner edge, so it is thrown in folds. The margin, which in the normal state lies at the surface of the body, stains deeply like a stout filament. The trailing flagellum lies usually more or less parallel to this.

Above the middle part of the anteromedial edge of the cresta of the specimen from which figure 1, plate 17 was drawn, there was a short, deep-stained, finger-shaped structure. This appeared to be an appendage of the cresta, originating from the edge, curved, and directed anteriorly. It was not seen on any other specimens, so it evidently is not characteristic.

The trunk of the axostyle consists of an outer membrane, a core that may be

stained by iron-haematoxylin, and a clear zone between the two. As was noted above, in the specimens that Dogiel identified as *G. herculea* the axostyle was colorless after iron-haematoxylin staining; but in those which he classified as *M. polymorpha* he described the stainable core. However, I have observed no difference between the two forms in the axostyle structure, and I think that the apparent difference was a peculiarity of Dogiel's preparations, which did not reveal the axostyle very well in the flagellated forms. The markedly spindle-shaped form that Dogiel described for the trunk of the axostyle of *G. herculea* does not agree with my material. The trunk of the axostyle is stout, 2½$\mu$ in the specimen of figure 1, plate 17, and its diameter is nearly the same from behind the nucleus to near the posterior end. In some forms it appears somewhat narrower near the nucleus than more posteriorly, but I have never seen it in so markedly a spindle form as in Dogiel's figure 10, plate 17 (fig. A, 1). The core, however, is thick in its middle part and has a considerable taper anteriorly and posteriorly. Where the core is slender, the clear part is broader; the shape as shown by Dogiel may be due in part to his failure to observe the outer structure. I have seen nothing to indicate that the axostyle is composed of fibrils, as Dogiel described it.

The posterior end of the axostyle is sharpened to a point. In the specimen of figure 1, plate 17 the posterior end of the axostyle is truncate, but that is not typical of the species. In my slide preparations the axostyle was usually enclosed in the cytoplasm, and I did not find the rather long projecting part which Dogiel described. The projecting spike appeared to be normal, however, in the living specimens which I observed (fig. B, 1).

Alongside the nucleus—on the right side if the cresta is considered to be dorsal—the axostyle is expanded in a capitulum (pl. 18, fig. 6). It is flattened, broadened, then narrowed in extension toward the basal granules. It is a relatively simple capitulum, but it has a special feature in a posterior armlike appendage, comparable to what exists in certain large devescovinins. In figure 6, plate 18, the anterior part of this extension appears as a ridge elevated above the capitular membrane. The deep-stained fibril on the observer's left of the capitulum of plate 17, figure 1 appears to be an edge of this elevation. Posteriorly there is a protuberance that becomes free of the rest of the capitulum and bends around the posterior end of the nucleus. The end of this arm, which is rounded in cross section, is in contact with the nuclear membrane on the left side. Thus it is in a position to function in holding the nucleus in place, in close relation to the head of the axostyle. The nucleus, axostyle, cresta, and flagella constitute a unified group, and the relationship of these organelles is not altered in mastigonts isolated from the cytosome (pl. 18, fig. 3).

In fixed material, the membrane of the nucleus is usually separated by a narrow clear space from the central chromatin mass (pl. 18, figs. 2, 3). In that mass there are numerous close-set small granules, and a variable number of larger spherules. The latter retain the stain more tenaciously than the chromatin granules. Dogiel's material was probably too much destained to show the smaller granules, so that his attention was directed chiefly to the few larger ones, and he mistakenly supposed that the nucleus is very poor in chromatin.

Dogiel devoted much attention to the supposed discharge of chromatin from the nucleus into the cytoplasm. He wrote about a constant oozing from the nucleus of substance stainable by iron-haematoxylin, through a curious spastic action of the nuclear surface. The extruded substance was said to form a film against the nuclear membrane; this was supposed to thicken and eventually separate in the form of an oval body, which migrated into the posterior cytoplasm. I made no observations on my material that conform with this account, and I think that Dogiel misinterpreted what were actually stainable cytoplasmic inclusions or mastigont structures in contact with the nuclear membrane. As in most of the accounts of supposed extrusion of chromatin through the nuclear membrane, the observations made seem to have been arbitrarily forced into a particular framework of hypothesis, and do not constitute proof of that hypothesis.

Dogiel described a "slightly developed cytostome" in *G. herculea*. In this account, again—and I believe that the same is true in all reports of supposed cytostomes in trichomonad flagellates—observations have been wrongly interpreted. I found no structure in *Gigantomonas* which I would regard as a cytostome; and Dogiel's structure lies in the same position as the anterior extension of the capitulum of the axostyle, which he did not recognize as such. Pereira and de Almeida (1943) denied the existence of a cytostome in trichomonads, and called attention to errors of interpretation, in this and other supposed features, that have been made in studies of that group of flagellates. No cytostome exists in Devescovininae, and I have not found it in any flagellates of the *Trichomonas* group that I have recently examined. When I reported it in certain species in 1931 I was, in common with other protozoölogists at that time, under the influence of one of the unfortunate morphological preconceptions deplored by the Brazilian authors.

In the flagellated form of *Gigantomonas herculea* there are few foreign inclusions; when there are any at all they are only a few small fragments of plant material (pl. 18, fig. 2). Only one specimen was found in which the body was distorted by a large ingested fiber. Evidently plant material is ingested, but it does not accumulate in any notable amount. Rounded chromatic masses are sometimes present in the cytosome (pl. 17, fig. 1); these may be residues of food material. Dogiel wrote of a ventral chromidium as a permanent inclusion in the middle part of the body. This was shown as a granular or spongy mass of irregular outline and sometimes, instead of one larger mass, there were two or three smaller ones. I have seen nothing in my specimens which could be regarded as a chromidium. It is most likely that Dogiel's structure was a *Sphaerita*-like cytosomal symbiote, which happened to be present in all the specimens he observed. Such organisms occur frequently in devescovinins, and I have observed that they were sometimes present in almost all specimens of a faunule.

In what seem to be the best preserved specimens of the flagellated form, there is no special differentiation of the peripheral region of the cytosome. One sort of cytoplasm, with granules throughout, extends to the surface layer (pl. 17, fig. 1). In specimens of altered form a differentiated outer zone of

cytoplasm may be developed (pl. 18, fig. 2). It is more or less homogeneous, and it varies much in thickness in different specimens and in different parts of the same specimen. It is to the differentiation of this zone that Dogiel referred in writing of ectoplasm and endoplasm. He stated that *Gigantomonas herculea* has a constant shape, without differentiation into ectoplasm and endoplasm. He was able to state this because he put into that species only the forms with unaltered shape and cytoplasm. The others he separated as type A of *Myxomonas polymorpha*. He noted that in type A the "ectoplasmic layer" is thick, dense, and more or less homogeneous; that the axostyle does not penetrate it; and that in specimens fixed upon cover glasses the ectoplasm may seem to have a still greater consistency and to separate some from the endoplasm.

The specimen of figure 11, plate 20 seems to be a degeneratively altered form. All gradations between it and the normal form can be found. In it all mastigont structures, including the anterior flagella, are enclosed in the interior, granular cytoplasm. An outer layer and underlying clear space are in some organisms differentiated in one part of the body but not in another part. The structure shown by this figure corresponds to what has been described as a cyst in some trichomonad and other flagellates; but it seems certain to me that it has nothing to do with encystation, which probably does not occur in *Gigantomonas*.

It is obvious from the above description that the forms which Dogiel designated by three epithets—*Gigantomonas herculea*, type A of *Myxomonas polymorpha*, and type B of *M. polymorpha*—all represent the same flagellate. It is difficult to comprehend the differentiation that he made, and it is understandable that he could not give any clear-cut diagnoses. His distinctions were in part based on: first, alteration of body form in making the preparations; second, detachment of the trailing flagellum in making the preparations; third, fortuitous variations in reaction of the axostyle to iron-haematoxylin; fourth, failure to observe the axostyle in some specimens ("type B of *Myxomonas*"); and, last, failure to account for obscurity or occasional loss of flagella.

Like Dogiel, I have not observed any division figures in the typical flagellate form. In *Gigantomonas herculea*, nuclear division seems to take place only in the modified amoeboid forms.

### The Amoeboid Forms and Division Stages

(Pl. 19, figs. 9–10; pl. 20, figs. 12–14; pls. 21–23, figs. 15–26; figs. C, D; fig. E, 3–10; fig. F)

The group of forms here to be described includes all those Dogiel put in *Myxomonas polymorpha* except his types A and B, which are considered in the preceding section. Although some amoeboid forms are comparable in size to the flagellated form, most of them are much larger. Some are spherical but most are elongate. One nearly spherical specimen had a diameter of 120$\mu$; another measured 180 × 170$\mu$. Sixty-five specimens of the amoeboid forms ranged in length from 75 to 360$\mu$, in width from 40 to 120$\mu$. Dogiel found lengths from 50 to 180$\mu$, widths from 40 to 90$\mu$. Evidently when *Gigantomonas*

*herculea* enters upon this amoeboid, dividing phase, it undergoes a marked increase in body size. Most of the division stages with typical reorganizing mastigonts ranged from 75 × 58$\mu$ to 160 × 120$\mu$. Some specimens exceeded 160$\mu$ in length; two of these measured 200$\mu$ and 300$\mu$, but they were relatively narrow, the width being 40 to 60$\mu$. The largest specimens were among the multinucleate types and those in which the division process had not followed the typical course.

Many of these amoeboid forms contain a large amount of ingested material; in some (pl. 23, fig. 23) the cytoplasm is densely packed with inclusions of vegetable matter. Dogiel also noted this difference between the amount of ingesta in the ordinary flagellate form and the dividing forms; in a hundred dividing forms of type D he found only one specimen without food inclusions. Most of the inclusions are small, and like Dogiel I have found that usually they lie directly in the endoplasm, with no surrounding vacuole. In some specimens long fibers are enclosed. The binucleate amoeboid form shown in figure F, 6, enclosed one end of a stout fiber 500$\mu$ long in a body 275$\mu$ long. Occasionally, small flagellates are enclosed in vacuoles; but ingestion of other protozoa appears to be exceptional.

Division as manifested in individuals of what Dogiel designated type D evidently follows the growth of the flagellate form and its transformation into an amoeboid phase. In the course of this transformation the mastigont structures redifferentiate. The flagella are lost; the cresta and axostyle are resorbed. Unfortunately I, like Dogiel, have been unable to find any specimens showing the intermediate stages of disappearance of the mastigont structures. In devescovinins the trunk of the axostyle is not resorbed until rather late in division, but in *G. herculea* it disappears at the beginning of division.

In the earliest division stage seen, the only extranuclear structure that is associated with the mastigont system is a small paradesmose. I have been unable to find the earliest phases in development of this structure; the shortest paradesmose that I saw had a length of 12$\mu$ (fig. C, 1). The strand is applied closely for its entire length to the membrane of the spherical nucleus, which at this time usually has a diameter of 12 to 16$\mu$, and is larger than the nucleus of the interphase flagellate.

Throughout the mitotic process the nuclear membrane remains intact. The paradesmose is in contact with the membrane, but it is not at any time sunk in a groove, as in some devescovinin flagellates. The substance of the nucleus goes through a typical succession of phases. In the earliest phase, when the paradesmose is still short, little difference from the interphase condition appears (fig. C, 2). There are a number of deep-staining endosomal bodies of diverse sizes; and the remaining chromatic material, which stains less intensely, is in dispersed granules or, later, in a skein of strands (fig. C, 4). The strands subsequently appear as definitive filiform chromosomes, which take the form of **V**'s or **U**'s (fig. C, 8, 9). They commonly show an orientation toward the region of the paradesmose. In heavily stained material the anaphase chromosomes often appear fairly smooth and compact, but are generally irregular and heterogeneous. In lightly stained material the inner ends of the

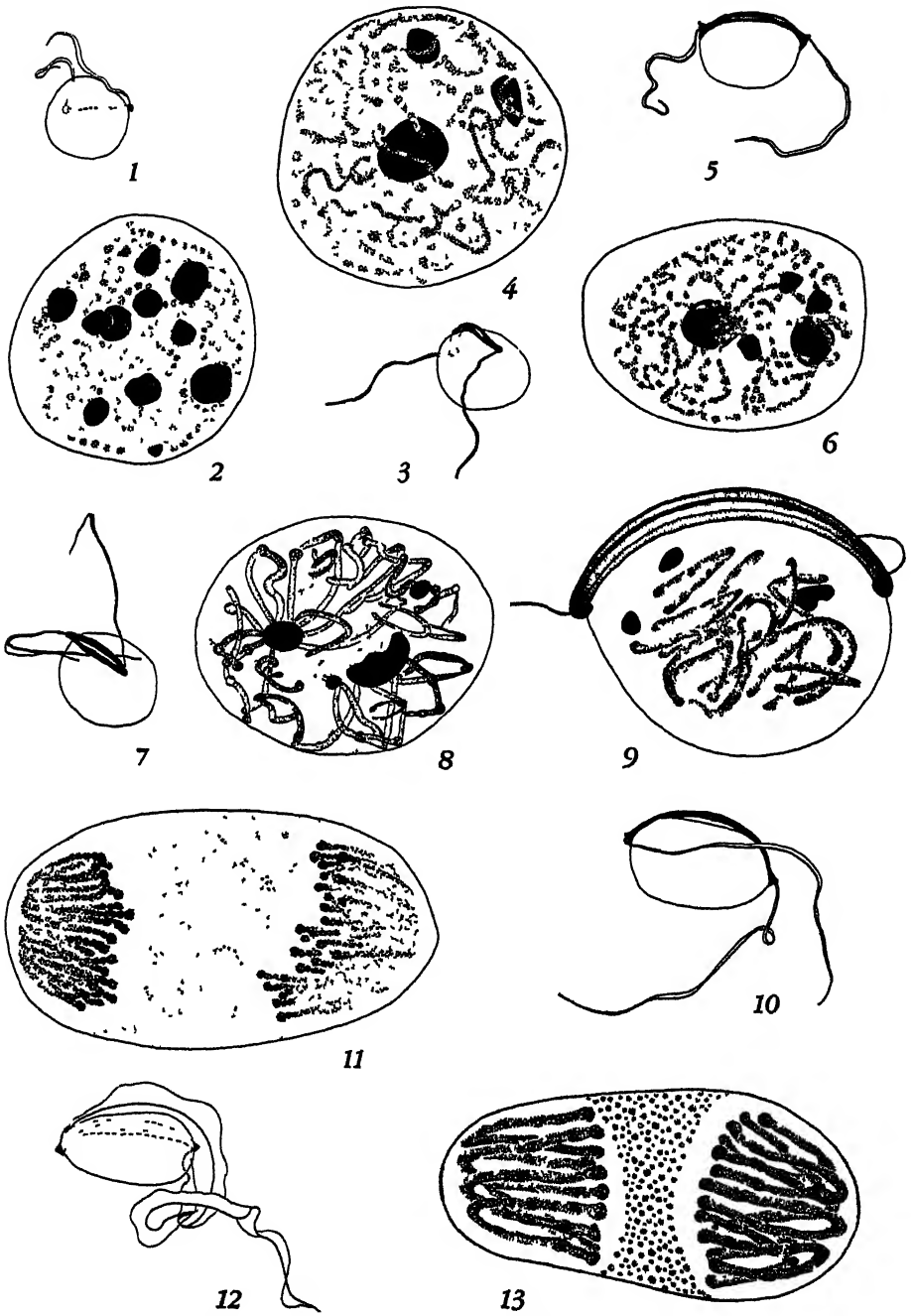


Fig. C. Division figures from amoeboid forms of *Gigantomonas herculea*. S.II. 1. Outline of nucleus, short paradesmose with deep-stained knobs at the ends, slender new crestas. The body containing this figure had dimensions of 110$\mu$ × 40$\mu$, the nuclear diameter 12$\mu$. × 960. 2. Nucleus of the specimen of fig. C, 1. There is little or no change from the interphase. × 2950. 3. Outline of nucleus, paradesmose, new crestas. Body 165$\mu$ long, 80$\mu$ wide at one part, 26$\mu$ wide at another. Nucleus 14$\mu$. × 960. 4. Nucleus of fig. C, 3. The chromatin is in well-defined

*(Legend continued at bottom of next page.)*

chromosomes often appear paler than the parts near the poles (fig. C, 11). In anaphases the arms of the V's are more or less straight rods, often about 4$\mu$ long (fig. C, 13). The apices of the V's are directed toward the polar ends of the nucleus; the preparations did not show any fibers between these apices and the region at the end of the paradesmose, but they probably exist. The chromosomes retain the V form throughout the anaphase and telophase, until the reorganization process is so far advanced that they cannot be traced. I have not been able to make convincing counts of the exact number of chromosomes. The number appears to be constant; it is probably not less than 15 or more than 18.

Endosomal material persists in the form of spherules until the anaphase. In that phase, between the two polar groups of chromosomes, there is interzonal granular material (fig. C, 13; D, 2). The endosomal spherules have by this time disappeared, so the interzonal material probably originates in the endosomal substance. If so, it loses as time goes on the capacity for intense iron-haematoxylin staining which characterizes the endosomal spherules; the interzonal substance stains less intensely than the chromosomes. This substance is still present after constriction into two nuclei, appearing in crescentic form, in optical section, at the side of the nucleus opposite the end of the paradesmose (fig. D, 4, 5). Later this material may become dispersed in the nucleus, but more probably it disappears. The endosomal material appears to arise in contact with the inner ends of the chromosomes.

In many specimens in which the nucleus is in an early prophase, a filament is found attached to each pole of the paradesmose (fig. C, 1, 3, 5). Dogiel saw thin curved threads extending from the ends (his fig. 30, anaphase) but did not understand their significance, though he suggested that they might represent the remains of the "undulating membrane" of the parent flagellate. That could not be true, because there are two filaments, one at each pole, but only one original "undulating membrane" associated with a single nucleus. The filaments evidently represent newly developing structures. The filament is at the border of a more lightly staining membrane—it often requires careful observation to see this at all—and successive stages of broadening of this membrane can be found in prophases and anaphases (fig. C, 1, 3, 5, 7, 10, 12).

*(Legend for fig C continued from preceding page)*
slender strands. × 2950. 5. Outline of nucleus, paradesmose with flat, expanded ends, new crestas. Body 100$\mu$×48$\mu$, nucleus 14$\mu$×9$\mu$. ×960. 6. Nucleus of the specimen of fig. C, 5. Within it the chromatin is in varicose strands, many of which are oriented toward the part of the membrane adjacent to the paradesmose. × 2950. 7. Outline of nucleus, paradesmose, new crestas. Body 120$\mu$ long. × 960. 8. Nucleus of fig. C, 7. The chromosomes are in the form of V- and U-shaped bodies; endosomes still present. × 2700. 9. Prophase nucleus and fibrillar paradesmose, with deep-stained, somewhat enlarged ends. The new crestas are well developed; their attachment is shown but the full length is not represented. Only a few of the chromosomes are shown. × 2950. 10. Outline of late anaphase nucleus, paradesmose, and new crestas. The enlarged, triangular, deep-stained ends of the paradesmose are applied to the poles of the nucleus at the place toward which the chromosomes converge. × 960. 11. Nucleus of fig. C, 10. The middle zone of the nucleus is occupied by a granular interzonal substance. × 2950. 12. The new crestas are long, and are broader than in preceding specimens. × 960. 13. Nucleus of fig. C, 12. The interzonal substance is in deep-stained granules, which are massed in the form of a biconcave disc between the groups of chromosomes. Only a few of the U-shaped chromosomes that are present in this nucleus are shown. × 2950.

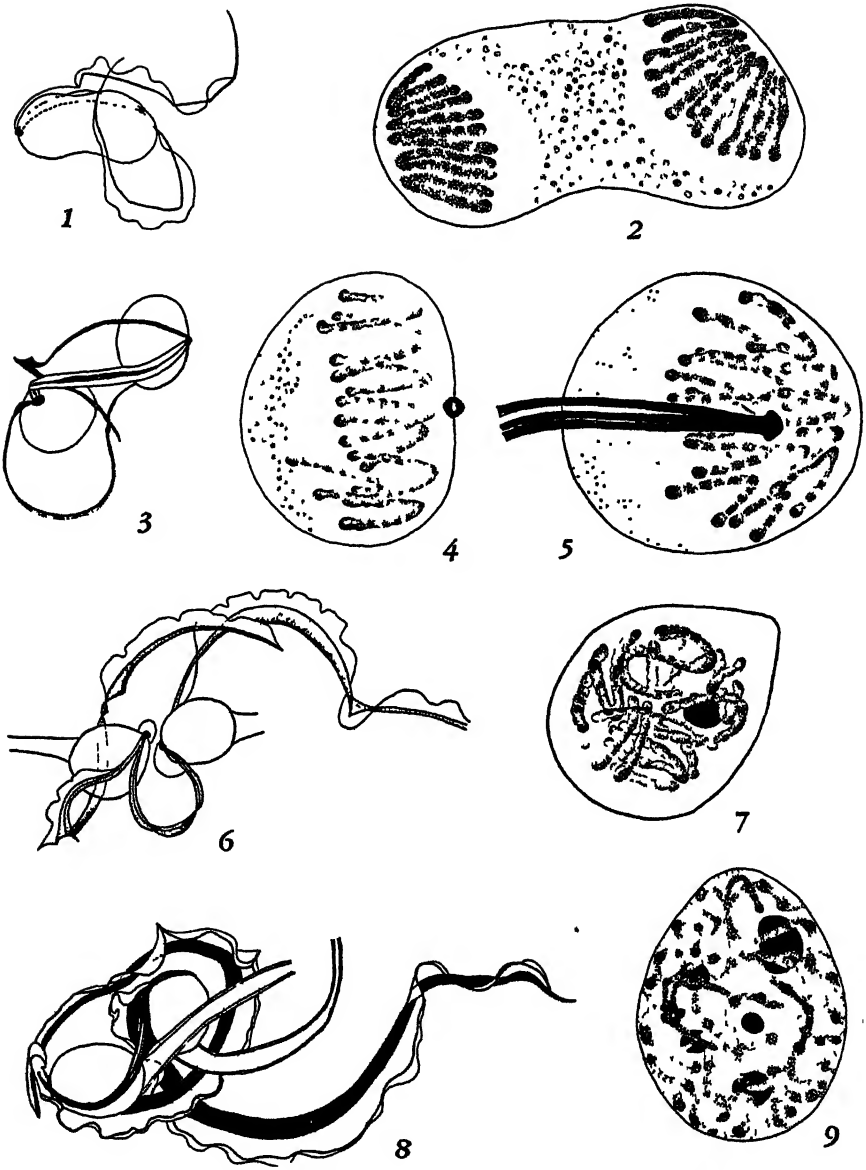


Fig. D. Division figures from amoeboid forms of *Gigantomonas herculea*, continuation of fig. C. S.H. 1. Outline of late anaphase nucleus, well-developed new crestas. × 960. 2. Nucleus of fig. D, 1. The chromosomes are V-shaped. × 2950. 3. The paradesmose seems to consist of 3 fibrils, and it ends in large, deep-stained granules. The new crestas are long. The body containing this figure measures $100 \times 50\mu$, each nucleus is about $9\mu \times 12\mu$. × 960. 4. Diagram of the right-hand nucleus of fig. D, 3. The large granule at the end of the paradesmose is shown. The granular interzonal substance is at the opposite side of the nucleus. Only part of the number of V-shaped chromosomes are represented. × 2950. 5. Telophase nucleus containing V-shaped chromosomes, only a part of which are shown, and interzonal substance. The part of the fibrillar paradesmose, with dense enlarged end, that lies over the nucleus is shown. × 2950. 6. New axostyles have developed, and in the region of the ends of the paradesmose is part of the rounded new capitulum. New crestas are advanced in development. The body containing this figure is $165\mu \times 100\mu$, the nuclei $10\mu \times 11\mu$. × 960. 7. Nucleus of fig. D, 6.

*(Legend continued at bottom of next page)*

After nuclear division has been completed, and the nuclei reorganized, a fully developed cresta may be found associated with each (pl. 21, figs. 15, 16). The filaments in the prophase, then, are developing new crestas, as indicated by a complete succession of forms. Dogiel recognized the new "undulating membranes" in later stages, but he did not have available for study enough intermediate phases to study their origin. He considered, therefore, that the new "undulating membranes" do not begin to form until after the two daughter nuclei are produced. In this account he was hampered by confusion of two different series of development, as is explained farther on in this article.

The ends of the paradesmose stain deeply with Heidenhain's iron-haematoxylin. When observed from above, on the upper surface of the nucleus, they appear as rounded knobs (fig. C, 3; D, 5; pl. 19, fig. 9); when observed from the side, in the same plane as the nucleus, they often show a triangular form (fig. C, 5). The paradesmose between them is sometimes stained as a compact, homogeneous-appearing strand, and the terminal enlargements grade into the remainder without any distinction. More often, however, the paradesmose shows a fibrillar structure (fig. C, 7, 9). Between these compact ends are several fibrils; commonly 3, 4, or 5 can be distinguished. The fibrils vary in their degree of separation; they may be so close together that no space shows between them, or they may be separated by clear spaces of varied extent. Occasionally one of the fibrils is far from the group of the others (fig. F, 2).

In the prophase, or at least in the earlier part of it, there are no separate granules at the ends of the paradesmose (fig. C, 1, 3, 5, 7; E, 6). Each new cresta then meets directly the structure at the end of the paradesmose at the outer angle of the triangle (fig. C, 5). In the anaphase and later stages granules are present separate from the ends, but very close to them, and to these granules cresta and flagella are attached (fig. E, 4–7). Sometimes only one of these granules can be made out, but often there are distinctly two. When the stain of the paradesmose is pale, except for the black ends, each end may appear as a third and much larger granule (fig. C, 5). When the paradesmose has disappeared, and also in interphase flagellates, there is a large granule in the same relation to the other granules, and to it neither cresta nor flagella attach. The large granule may be the persisting structure at the end of the paradesmose, and may perhaps be regarded as a centriole. That seems likely. The observations that I have made suggest it, but do not prove it; they have not excluded the possibility that the large granule in the interphase has been differentiated after the paradesmose, with its terminal structures, has disappeared.

The paradesmose elongates as the nuclear changes proceed. At the time just before constriction of the elongated nucleus, it is applied to the mem-

*(Legend for fig. D continued from preceding page)*
A new endosome has appeared. The chromosome outlines are still apparent, some showing V form, but they are not so definite as in the anaphase. × 2950. 8. The new axostyles and new crestas are well developed; a trailing flagellum is present along the margin of the cresta. The cresta shows differential staining in the black basal part and clearer outer part. × 960. 9. Nucleus from fig. D, 8. Endosomes have developed and the chromatin is in diffusely arranged strands. × 2950.

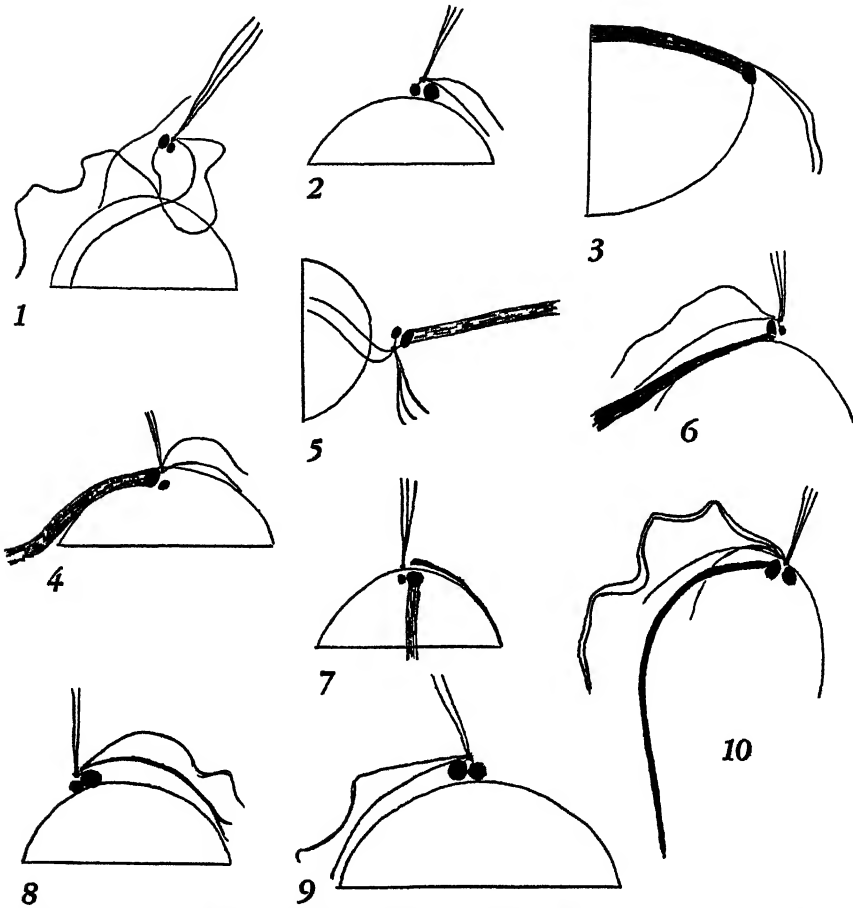


Fig. E. Freehand diagrams showing the granules in the region of origin of the flagella and cresta in *Gigantomonas herculea*. Except in prophases and early anaphases, there are two large granules, unequal or equal in size, situated close together on or near the anterior nuclear membrane or (especially in the flagellated form) at some distance from it. Neither of these granules is met by the flagella or cresta, but one of them appears at the end of the paradesmose in stages where that structure is present. Anterior and close to these two granules is a less well-defined and usually less critically stained small granule or, as it occasionally appears, pair of granules close together. This body gives origin to flagella and cresta, and there are indications of connecting filaments between it and the two large granules. 1. Flagellated form, similar to pl. 17, fig. 1. The cresta is large, and the anterior extension of the capitulum of the axostyle is indicated in the diagram. Granules like the two unequal large ones are shown also in the specimen of pl. 17, fig. 1. 2. Flagellate in an incomplete state of development, granules in similar grouping closer to nucleus. 3. Nucleus in late prophase, with paradesmose. At the end of the paradesmose, a deep-stained body is set off similar to the larger of the granules of the interphase. The specimen is similar to those of fig. C, 5–8. As is shown also in those figures, the new cresta directly meets the body at the end of the paradesmose, at its anterior part. At least part of the basal-granule complex of the interphase has been dedifferentiated. 4. An amoeboid form with paradesmose and moderate-sized new crestas, similar to fig. X, 4. Adjacent to the body at the end of the paradesmose is a smaller granule, and flagella and cresta join a separate granule. Comparable to the interphase structure, except that the paradesmose meets one of the granules. 5, 6. Similar forms with a similar situation of the paradesmose. In 5 there are indications of connecting fibrils from the flagellar granule to the two large granules. 7. Binucleate amoeboid body, with paradesmose. The new crestas are very small, as in pl. 23, fig. 20. 8, 9. Amoeboid body with one nucleus and no paradesmose. The arrangement of granules is the same as when the

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brane and has a length equal to the diameter of the two future daughter nuclei (fig. D, 1). It continues to elongate after formation of the daughter nuclei, and its dimensions show that it is at first growing by increase of substance, not merely being drawn out. The nuclei separate and the paradesmose may extend straight between them, but very often it is bent in a U form (fig. D, 6). Later the paradesmose becomes more slender as it lengthens (pl. 20, fig. 13). In later stages with two reorganized mastigonts it may be very long: in one specimen it measured 120$\mu$. This elongation of the paradesmose is like that which has been found to take place in many trichomonad flagellates.

New flagella have developed during the process of division, and in stages where there are reorganized nuclei, both the anterior flagella and the trailing flagellum can be found (pl. 21, figs. 15, 16). The anterior flagella may be imbedded in the cytoplasm at least for part of their length, and the trailing flagellum parallels the margin of the cresta and with it may lie deep in the cytoplasm.

New axostyles are differentiated later than the crestas; no evidence of them has been found before the later telophase stages. The capitulum seems to begin to develop first, and the part of it that extends anterior to the nucleus can sometimes be seen when no other axostylar structures are visible. The ends of the paradesmose and associated granules are in close relation to this extension of the capitulum. In binucleate stages, where there are well-developed crestas and paradesmose, the new axostyle trunks are present (pl. 21, fig. 16; fig. D, 8). At first no relation in position exists between these trunks and the paradesmose, but later that elongated strand may come to lie parallel to the axostyles, or may even spiral around them. This position is a consequence of the later orientation of mastigont structures; the two structures are entirely independent in their origin and development.

Some specimens have been found with two completely reorganized mastigonts but no paradesmose, which had evidently been resorbed before division of the cytosome (pl. 20, fig. 14). I have found no stages of actual division of the cytosome; it no doubt takes place by distribution of the cytoplasm between the two mastigonts, and each daughter structure is again the typical flagellate form of *Gigantomonas herculea*.

Two interesting abnormalities encountered in study of this series of forms deserve special attention. One of them was a bimastigont form with a paradesmose, but in one of the mastigonts, otherwise intact, the nucleus was absent (pl. 21, fig. 16). It was not found anywhere else in the cytosome, which was only slightly ruptured, so it does not seem likely that it was displaced mechanically in making the smear. Possibly there had been an abortive division in which two daughter nuclei were not produced, although each end of the paradesmose gave origin to its set of extranuclear structures.

The other specimen contained a quadripolar division figure, with four

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*(Legend for fig. E continued from preceding page.)*
paradesmose is present, if the deep-stained body at the paradesmose end is recognized as one of these. In 9 the two large granules are of nearly equal size. 10. A one-mastigont flagellate, large cresta. A fibril that represents a persisting part of the paradesmose is present, meeting one of the two large granules which are of nearly equal size.

anaphase groups of chromosomes (pl. 19, fig. 10). There were four paradesmoses; not all of the poles were interconnected. Three of the poles were interconnected by three paradesmoses arranged in a triangle. To the fourth pole a single paradesmose was attached. Its position was abnormal, however; instead of meeting one of the other poles, it extended free in the cytoplasm. A cresta was attached to its free end, as well as to the other end. Of the three poles interconnected by the triangular group of three paradesmoses, two bore crestas. The other, being without a cresta, seemed to be the one from which the free end of the paradesmose had been separated. It seems unlikely, however, that this unusual position of the free paradesmose and cresta was a result of mechanical damage in making the smear. The large amoeboid body was intact, with no apparent rupture, and the groups of chromosomes within the nucleus were not disturbed. There was one common deep-stained enlargement and group of astral rays at each of the poles of the triangle, and a similar enlargement and group of astral rays marked the end of the free paradesmose. This would probably not be true if there had been a simple, sudden rupture. The structure may have grown free while the organism was alive and undisturbed. Each of the four groups of chromosomes in this multipolar division figure consisted of as many as in the two groups of the normal bipolar figure.

The series of events described for *Gigantomonas herculea* in the preceding account of division agrees in general with the usual mitotic division and reorganization of mastigonts in trichomonad flagellates. There are, however, many forms which do not fit into that series. One may think that the reason they do not fit is that they belong to a different organism. Dogiel adopted that concept; but since he found so much resemblance to forms that I have now shown to be dividing *G. herculea,* he was unable to draw a sharp separation, and he included the dividing *Gigantomonas* with the supposedly different organism. No sharp lines of separation can properly be drawn anywhere in the group, so I have treated the different forms here as all belonging to the one flagellate, which follows somewhat divergent courses in different phases of its life history. It is possible, I recognize, that this view is not correct, and that actually two or more organisms are involved. The status of the flagellates cannot be definitively established without a much larger series of forms than I have been able to study. My account will, I hope, eventually be corrected and supplemented by an investigator who has unrestricted access to the termites that contain this interesting symbiote.

A count was made of the different forms encountered in succession in a survey of a number of slides from *Hodotermes mossambicus.* Of 300 specimens, 121 were interphase flagellate forms and 37 belonged in the typical division series. The remaining 142, about half of the total, almost all showed evidence of being in a division stage or having recently divided. They are considered here instead of in the foregoing account of division because of differences of several kinds from what may be regarded as the typical division series in trichomonad flagellates. Yet they must be considered in connection with division, with a modification of one sort or another.

Of the 142, 127 were binucleate specimens. In almost all of them the nuclei were in an interphase. A rounded endosomal body of moderate size was often present in the center of the nucleus (pl. 22, figs. 18–22; fig. F, 3, 5). Sometimes there were several endosomal bodies in addition to, or instead of, this central one (pl. 21, fig. 17, upper nucleus). The chromatic material stained more lightly than the endosome with iron-haematoxylin, and was organized in granules or strands (fig. F, 3, 5). With regard to the extranuclear structures, there were several sorts of organization. In all but 14 of these 127 amoeboid specimens a paradesmose, which consisted of several fibrils, connected the two nuclei (pl. 23, fig. 23). Close to each end of the paradesmose were two small deep-stained granules of approximately equal size—the basal granules of the mastigont structures. In the few binucleates which lacked a paradesmose (pl. 23, fig. 24), it had evidently been resorbed. The specimens showed a marked difference from the typical division series in regard to the cresta and axostyle.

In the typical division series, in agreement with other trichomonads, both cresta and axostyle develop early and are advanced in growth by the time the nuclei have reorganized. In about a quarter of these atypical amoeboid specimens neither cresta nor axostyle was present. Another group of 25 of them had no cresta, but the axostyle was represented in some degree. In the greater number of these binucleate amoeboid specimens, close observation showed a small membranous structure anterior to the nucleus; that structure apparently belongs to the capitulum of the axostyle (pl. 21, fig. 17; pl. 22, fig. 18; fig. F, 1, 4). Many of the specimens that seem to lack any axostylar structures probably do have the capitular structure, but are either oriented in such a way or are stained in such a manner that it cannot be seen. The basal granules near the end of the paradesmose rest upon this membrane. The trunk of the axostyle is developed in some specimens (pl. 22, figs. 18, 20). Only a few were found in which the trunk was stained so as to show very clearly, but in many others it is evidently present though difficult to see. The structure is slender; generally it appears more slender than in the typical division series.

Fifty-six of the 113 binucleate specimens with a paradesmose and three of those without it had crestas in some state of development. In some the crestas appear as short, deep-stained filaments that lie close to the nuclear membrane (pl. 22, figs. 21, 22). It is unusual that these do not extend out free in the cytoplasm as do the crestas in the typical division series. Another difference from that series is that expansion may take place before the filament has become so long. Thus crestas appear that are short and relatively broad—much like miniatures of the full grown cresta (fig. F, 2). In other specimens the crestas approach more closely to the more usual, elongate form (pl. 22, fig. 20).

Three long anterior flagella are undoubtedly present in at least some of these amoeboid specimens (pl. 22, fig. 18; fig. F, 1). Those in which I have seen them have lacked the cresta, and also, apparently, a trailing flagellum. Only occasionally have I been successful in observing the full length of the anterior flagella; generally only the basal part can be made out and most often, of

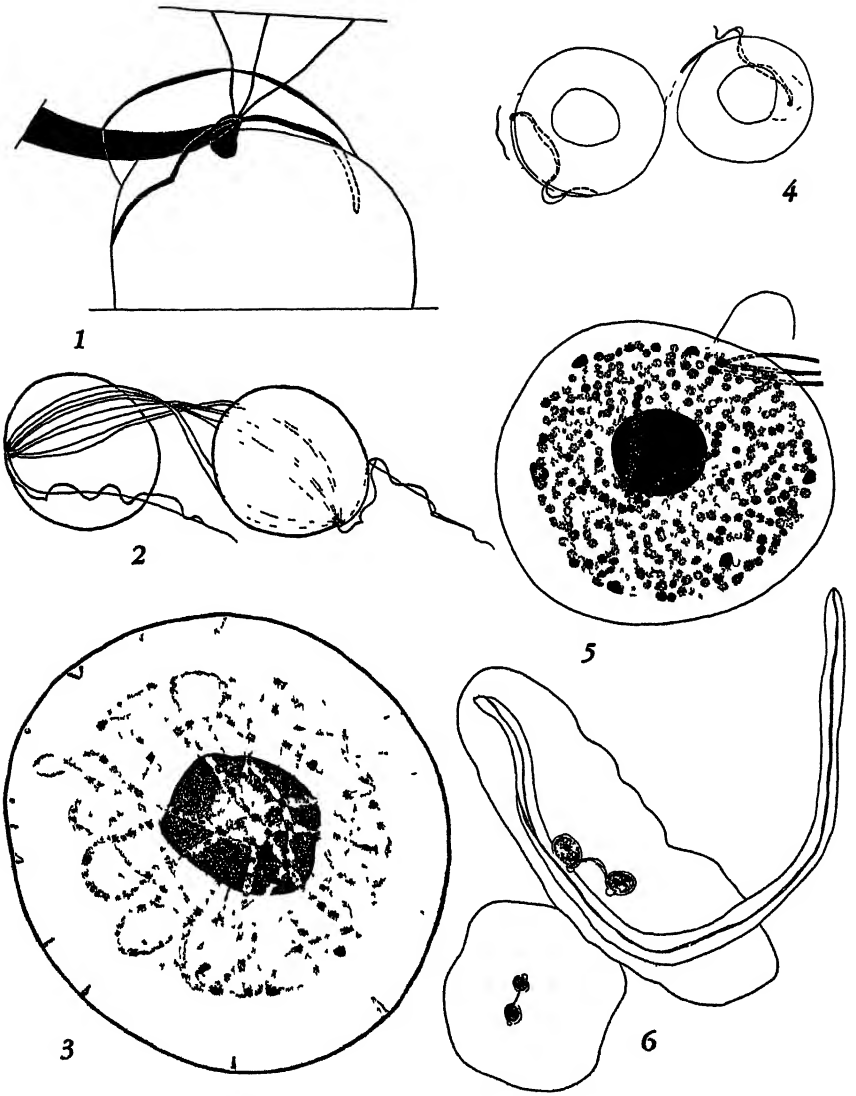


Fig. F. *Gigantomonas herculea.* (1. From *Microhodotermes viator.* 2–6. From *Hodotermes mossambicus.*) 1. Detail from a large, binucleate, amoeboid specimen. The figure shows part of the nucleus in outline, anterior to this part of the capitulum of the axostyle, three anterior flagella, a large granule at the place of origin of these and the stout black paradesmose, a filament passing to the left and applied to the nuclear membrane (nuclear rhizoplast), and a filament to the right along the nucleus that possibly is a small new cresta. S.H. × 2950. 2. Division figure in a large amoeboid form. The fibrils of the paradesmose are widely separated, except in the central part and at the ends. Nuclear diameter 22$\mu$. The new crestas are rather short and slender, in contrast to the large size of the crestas at this stage in the typical division series. S.H. × 960. 3. One nucleus of fig. D, 2. There is a large central nucleolus, around which the chromatin is arranged in strands. S.H. × 2950. 4. A pair of nuclei from a large amoeboid form about 250$\mu$ × 125$\mu$. Two pairs of nuclei are present in the specimen. Each has a large central nucleolus, and associated with each is a small capitular membrane and a relatively short new cresta. Nuclei 21$\mu$ and 18$\mu$. One of the nuclei is shown in detail in pl. 22, fig. 19. S.H. × 960. 5. One of the nuclei of the pair in pl. 21, fig. 17. At the end of the paradesmose, which is represented by 3 fibrils, are 2 granules. A part of the capitular mem-

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course, the structure cannot be seen at all. The flagella connect with the basal granules near the ends of the paradesmose.

Among the 142 amoeboid specimens, ten were uninucleate (pl. 23, fig. 25). These were similar to the binucleates in respect to the variability in the presence of other organelles. A few had a remnant of part of the paradesmose; all had the membranous extension of the capitulum but not all showed the trunk of the axostyle; some had no cresta, some a miniature cresta, some a cresta of the more usual size and shape.

Multinucleate specimens are not infrequent among the large amoeboid forms. Several specimens were found with 4 and 6 nuclei. They showed the same variability as did the other amoeboid forms in the presence of crestas and axostyles, though the anterior capitular membrane, and almost always the paradesmose, was present. On a slide made from *Microhodotermes viator* there were several unusual specimens with cytosomes of large size and with from 25 to 40 nuclei (pl. 23, fig. 26). The nuclei were small in size, with a diameter of about 8$\mu$. Each was accompanied by a small capitular membrane (pl. 23, fig. 27) and basal granules were upon this, but there was no evidence of any paradesmose, cresta, or trunk of the axostyle.

These often large amoeboid forms, which as we have seen are at least sometimes provided with flagella, usually contain many ingested particles (pl. 23, figs. 23–25). Often the cytosome is packed with fragments of plant material, and long fibers may be enclosed (fig. F, 6). The amoeboid forms are evidently voracious feeders.

## SYSTEMATIC POSITION OF GIGANTOMONAS

*Gigantomonas* belongs to the group of flagellates that have a mastigont system of the sort which we may designate as the trichomonad type. This type of system is characterized by an axostyle which passes longitudinally through the body and is associated anteriorly with the single nucleus, usually by a true parabasal body situated dorsal to the nucleus, by a number of anterior flagella that arise in one group of blepharoplasts, by a trailing flagellum which may or may not adhere to the surface of the body, and by the existence in mitosis of a paradesmose that is usually applied to the surface of the dividing nucleus. They have, thus, a uniform type of organization in which they are distinct from many other flagellates, and they show a degree of uniformity in division. This group of flagellates can appropriately be designated as the trichomonad group, since *Trichomonas* is the oldest contained genus.

The limits of the group must, however, be drawn differently from those of the family Trichomonadidae as it is considered by Wenyon (1926), some of his genera being excluded. The family Trichomonadidae as given by Kudo (1946) contains only genera that would be considered here to belong in the

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*(Legend for fig. F continued from preceding page)*
brane is shown. S.H. × 2950. 6. Two specimens represented as they occur together. The largest one is 275$\mu$ × 80$\mu$, the other 130$\mu$ × 120$\mu$. The larger organism has ingested part of a very long plant fiber. Each specimen contains a pair of nuclei at the same stage in development as those in the other, but they differ greatly in size. In one the nuclei are 15$\mu$ × 20$\mu$, in the other 11$\mu$ in diameter.

group, but in addition other genera should also be included according to my concept of the group. The existence of an undulating membrane, on the basis of which Kudo defined the group, is not so fundamental as certain other features of organization. *Monocercomonas* (*Eutrichomastix*), for instance, has a mastigont system and a division process altogether comparable to that of *Trichomonas*, except that the trailing flagellum does not adhere in a membrane, and that there is no chromatic basal structure. It is also not reasonable to indicate too much of a dislocation between *Trichomonas* and the devescovinid flagellates, in which the trailing flagellum does adhere to the surface more or less, but forms no true membrane, and there is a chromatic basal structure. We find these flagellates, of what is designated the trichomonad group, in Kudo's families Polymastigidae, Devescovinidae, and Trichomonadidae.

Kirby (1931) considered the family Trichomonadidae, but included some genera that now are not admitted to belong there, namely, *Polymastix*, *Retortamonas*, and *Tetramastix*. The position of *Tetratrichomastix* is uncertain. Kirby proposed a subdivision of the family into three subfamilies. The Polymastiginae would not stand as included in the group; in its place is the Monocercomonadinae, with the genera *Monocercomonas* (*Eutrichomastix*) and *Hexamastix*. Probably, too, *Tricercomitus*, which has an adherent trailing flagellum but no true undulating membrane and no chromatic basal structure, should be put in the Monocercomonadinae. The second subfamily, Devescovininae, stands as given except for *Gigantomonas;* it includes *Janickiella*, which is a synonym of *Foaina*. The third subfamily, the Trichomonadinae, corresponds to the family Trichomonadidae as given in Kudo (1946).

Whether these subfamily groups should instead be designated separate families is, of course, a matter of choice. It has seemed to me that the marked unity in fundamental organization and the distinction from such groups as the Pyrsonymphidae, for instance, is best emphasized by the arrangement made. It would be desirable, if the subfamilies are made families, nevertheless to indicate their relationship to one another by a higher common grouping. The trichomonads as commonly understood are so closely related in organization of the mastigont system and in division to the hypermastigotes, that a common category might be established to include all of these flagellates.

The foregoing discussion of the arrangement of trichomonad flagellates has the purpose of building a framework within which to put *Gigantomonas*. This flagellate plainly belongs in the trichomonad group; that being understood, it may be considered whether it can be placed in the trichomonads proper, or in the devescovinids, or should have systematic category of its own.

It seems clear to me that *Gigantomonas* does not belong very close to *Trichomonas*, where the flagellate form of *G. herculea* has generally been placed. Reichenow (1928) wrote that it is a typical *Trichomonas*, differing only in its large size, repeating what Dogiel had stated when he described it. Both Reichenow and Dogiel supposed that there was a typical undulating membrane, and four anterior flagella of which one differed in size from the others. They failed to realize that the supposed large anterior flagellum is actually the trailing flagellum, which corresponds to the marginal flagellum

of the undulating membrane of *Trichomonas*. As I have shown in the preceding account, the structure said to be an undulating membrane is actually a cresta, which is comparable rather to the costa of *Trichomonas;* the outer margin of this cresta was mistaken for the marginal flagellum. The organization of *Gigantomonas,* then, differs as much from that of *Trichomonas* as does that of *Devescovina;* and it does not belong in the same systematic grouping as *Trichomonas* unless *Devescovina* is also in that grouping. It seems to me, however, that there are sound reasons for a separate grouping of the devescovinin flagellates.

Kirby (1931) listed *Gigantomonas* as one of the genera of the subfamily Devescovininae, placing it next to *Macrotrichomonas*. Connell (1932) not only included *Gigantomonas herculea* (together with its *Myxomonas* forms) in the subfamily Devescovininae, but he considered *Macrotrichomonas* to be a synonym of *Gigantomonas*. We have now to consider whether that synonymy is correct and, if not, whether *Gigantomonas* belongs in the subfamily.

The question of synonymy was discussed by Kirby (1942, p. 112), and the conclusion was reached that the two genera are distinct, in spite of much similarity in the flagellate phase. *Macrotrichomonas* contains seven known species, which all occur in the Kalotermitidae. They have hosts in several genera of that termite family, but have been found in no other group of termites. *Gigantomonas herculea* occurs in a group of termites, the Hodotermitinae, that is definitely separated from the Kalotermitidae. This suggests an evolutionary distinctness of the flagellates, in view of the close phylogenetic host relationships in termite protozoa. And when we consider the behavior and life history of *Gigantomonas,* that distinction is emphasized, although its structure—except for the absence of a parabasal body, which is not in itself a reason for generic distinction—corresponds closely to that of *Macrotrichomonas.*

All species of *Macrotrichomonas* have bodies that retain their form well in preparations, and show no alterations of form when examined in isotonic salt solutions. This is in marked contrast to what is true of *Gigantomonas* (p. 169). *Macrotrichomonas* divides in the flagellate phase, retaining the original flagella and developing new ones to complete the number, and making no significant change in size and activity. This again is in marked contrast to the great enlargement, the development of amoeboid forms, the loss of flagella, and the irregularities in morphogenesis in *Gigantomonas herculea.* Since in these respects the flagellate of *Hodotermes* is set apart not only from *Macrotrichomonas* but from all other devescovinin flagellates, it seems wise to retain at least generic distinction.

Differentiation of a somewhat comparable degree, though of a different character, exists in another group that has plainly developed from the devescovinin type of flagellate. The structure of the mastigont system in *Coronympha* and *Metacoronympha* is identical with that of the devescovinid flagellates. The only difference is in the polymastigont organization in the cytosome. Those flagellates, however, are customarily placed not only in a separate family, the Calonymphidae, but sometimes even in a separate sub-

order of the Polymastigida (Kudo: Polymonadina) or in a separate order of Mastigophora (Wenyon: Polymonadida).

In the features in which *Gigantomonas herculea* is distinct from *Macrotrichomonas*, it differs also from all members of the subfamily Devescovininae. Clearly, however, *Gigantomonas* has a close phylogenetic relationship with other devescovinid flagellates. It has differentiated in a manner distinct from all the rest, and has lost the parabasal body. Although its origin, like that of the Calonymphidae, is evidently within the devescovinids, or in a common ancestral flagellate, it seems desirable to recognize the degree of differentiation by a separate subfamily grouping. A new subfamily, the Gigantomonadinae, is accordingly proposed to contain it. The word Gigantomonadinae, for a division of the family Trichomonadidae, was published by me (1944, p. 364), but without any mention of its characteristics, of the type genus, or of included genera. As of that date, it may be regarded as a *nomen nudum*. The Gigantomonadinae and Devescovininae are closely related, and it is probably desirable to bring out this affinity by including them in one family, the Devescovinidae Poche; reserving the family name Trichomonadidae for *Trichomonas* and related forms.

Dogiel (1922) introduced the family name Amoebomitidae for the amoeboid *Myxomonas* forms, leaving the flagellate forms of *Gigantomonas* in the Tetramitidae. His diagnosis of the Amoebomitidae (translated from Russian) is: "uninucleate flagellates. The body has an amoeboid form. The locomotor apparatus is more or less reduced: the free flagella disappear, and sometimes also the axostyle, so that there is left only the undulating membrane (the dimensions of which, in comparison with those of the body, may be disproportionately small)." It is the existence of these amoeboid forms that is the reason for the separate grouping of *Gigantomonas*, so that his family name applies to the same category that I have named Gigantomonadinae, although its original contents and definition were not the same. The name Amoebomitidae is, of course, not formed in accordance with Article 4 of the International Rules of Zoölogical Nomenclature; therefore, no name similar to it can be used for the subfamily. When proposed, Dogiel's family contained only the genus *Myxomonas*, and the family name should have been based on the name of the type genus. The only genus in the subfamily Gigantomonadinae is *Gigantomonas*, of which *Myxomonas* is a synonym.

## DIAGNOSES

### **GIGANTOMONADINAE** subfam. nov.

Amoebomitidae Dogiel, 1922, Arkh. russk. protist. Obshch., 1:215.
Gigantomonadinae Kirby, 1944, J. Morph., 75:364 (*nomen nudum*).

*Type genus.*—*Gigantomonas* Dogiel.

*Diagnosis.*—Mastigont system of several anterior flagella, one trailing flagellum, axostyle, cresta within the cytoplasm, its edge at the surface; trailing flagellum paralleling and insecurely adherent to the outer edge of the cresta, but no undulating membrane; body of flagellate form very labile; an important phase in the life history is a much enlarged, amoeboid form, frequently binucleate with a persistent paradesmose, with reduced development of the mastigont structures.

## **Gigantomonas** Dogiel

*Gigantomonas* Dogiel, 1916, J. russe Zoöl., 1:6 (type species *G. herculea* Dogiel).—Connell, 1932, *pro parte*, Univ. Calif. Publ. Zool., 37:180.

*Myxomonas* Dogiel, 1916, J. russe Zoöl., 1:15 (type species *M. polymorpha* Dogiel).

*Diagnosis.*—Mastigont system with three long anterior flagella, one long trailing flagellum, large cresta, stout rodlike axostyle; typical trichomonad division series in amoeboid forms; atypical series of much enlarged amoeboid forms, generally binucleate with persistent paradesmose and reduced mastigont structures, differentiating this genus from *Macrotrichomonas*.

## **Gigantomonas herculea** Dogiel

(Pls. 17–23, figs. 1–27; figs. A, B, C, D, E, F)

*Gigantomonas herculea* Dogiel, 1916, J. russe Zoöl., 1:6, pl. 1, figs. 8–12.

*Myxomonas polymorpha* Dogiel. 1916, J. russe Zoöl., 1:15, pls. 1–4, figs. 13–44; text figs. 1–4.

*Type host.*—*Hodotermes mossambicus* Hagen. Africa.

Taveta, Kenya Colony. (V. A. Dogiel.)

T–3063. 72 mi. n. of Mbeya, Tanganyika Terr. (Xenosyntype slides TP–2060:10, 13.)
T–4017. Weenen, Natal. (Xenosyntype slides TP–2077:3, 4.)
T–4019. Weenen, Natal. (Xenosyntype slides TP–2075:9, 11, 18, 19.)
T–4082. Commadagga, Cape Province. (Xenosyntype slides TP–3000:6, 7, 12.)
T–4138. Near Calitzdorp, Cape Province. (Xenosyntype slides TP–3021:1, 6, 7, 8.)
T–4224. Near Kimberley, Cape Province. (Xenosyntype slides TP–3061:1, 2.)
T–4244. 28 mi. n. of Pretoria, Transvaal. (Xenosyntype slides TP–3070:1, 3, 5.)
T–4261. Near Bremersdorp, Swaziland. (Xenosyntype slides TP–3079:1.)

*Additional host.*—*Microhodotermes viator* (Latreille). Africa.

T–4070. Peddie, Cape Province. (Homosyntype slide TP–2096:5.)
T–4147. Near Barrydale, Cape Province. (Homosyntype slide TP–3027:4.)
T–4195. Near Dwyka, Cape Province. (Homosyntype slide TP–3050:8.)
T–4202. Near Victoria West, Cape Province. (Homosyntype slide TP–3052:6.)

*Diagnosis.*—Occurs in interphase flagellate form, amoeboid division forms, and enlarged, usually binucleate amoeboid forms with persistent paradesmose and incomplete mastigont systems. Interphase flagellate form: ovoid, rounded anteriorly, length about 60–80$\mu$, width about 35–50$\mu$; three slender anterior flagella 50–95$\mu$ long; trailing flagellum a rather slender cord about equal in length to the anterior flagella; cresta large, inner part flat and deep-staining, outer part relatively clear and undulated at margin, length of anteromedial edge 12–18$\mu$, posteromedial edge 49–60$\mu$, external edge 76–94$\mu$; no parabasal body; axostyle with moderately expanded capitulum, trunk stout, consisting of an outer membrane and a siderophile inner core, sharpened to a point posteriorly; nucleus 7–12$\mu$ × 5–10$\mu$, chromatin in small granules, endosomes several, moderate in size, varying in number and position; no adherent microörganisms; normal shape seldom retained in preparations. Amoeboid division forms: size 128 (80–300)$\mu$ × 63 (44–120)$\mu$; paradesmose fibrillar; in morphogenesis, complete reorganization of all cytosomal organelles. Binucleate and uninucleate amoeboid forms: size 175 (90–360)$\mu$ × 105 (65–170)$\mu$; nuclei 14.5 (7–22)$\mu$ × 13.5 (6–22)$\mu$; nucleus usually associated with a small capitular extension; other organelles of mastigont system incompletely differentiated; ingested material usually abundant in cytoplasm.

## DISCUSSION OF DIENTAMOEBA FRAGILIS

The most striking feature of *Dientamoeba fragilis* is the persistence of the binucleate state with an extranuclear desmose of which each end is applied to the membrane of one of the nuclei. In this particular, *Dientamoeba* is unlike any protozoan that has been described, except *Gigantomonas*. There are many

flagellates that have a binucleate or multinucleate condition but are without a persisting desmose; and there are many flagellates in which there is an extranuclear desmose, but this does not persist in a lasting binucleate state. It is, therefore, of particular interest that we find in *Gigantomonas herculea* another organism which frequently occurs in an amoeboid phase in which there are usually two nuclei between which a desmose persists.

Except for the absence of flagella, *Dientamoeba fragilis* has a close similarity to *Histomonas meleagridis,* according to Dobell (1940) and Wenrich (1944). *H. meleagridis* may occur in an amoeboid form without flagella. For the most part nuclear division is, as in flagellates in general, followed immediately by division of the body. An extranuclear desmose forms in nuclear division and persists until fission. In cultures, Bishop (1938) found a certain percentage of binucleate specimens, and in some of these (her fig. 11) the desmose is still present after the nuclei have resumed the interphase structure. But this state of development—binucleate with persisting desmose—is infrequent in *H. meleagridis* and in occurrence is to be compared with the occasional bodies of *Trichomonas vaginalis,* containing two or more mastigont systems, that sometimes occur in cultures (Trussel and Johnson, 1945) rather than with the normal state of *D. fragilis.*

In *Gigantomonas herculea,* however, the amoeboid binucleate form with persisting desmose is a prominent phase in the life history. In the high degree of development of the mastigont system of *G. herculea,* that organism cannot be compared with either *Histomonas* or *Dientamoeba.* But in those very features, which leave no doubt about its true systematic position, it may throw some light upon the doubtful taxonomy of *Dientamoeba.*

The desmose of *Dientamoeba* appears to be truly comparable to a paradesmose—the structure that in the monozoic Polymastigida is characteristic of dividing Trichomonadidae. It is evidently not simply a strand that is drawn out between two separating granules, but is a structure that has a capacity for autonomous growth, like the paradesmose of trichomonads. The thickness of the strand in telophase stages of *D. fragilis,* when its length is several times the nuclear diameter, appears in the figures by Dobell (1940) to be as great or greater than that of the short strand in prophase stages. That is true also of *Histomonas meleagridis,* so far as may be judged from Bishop's figures. The ends of the strand in these organisms, as in all trichomonad and hypermastigote flagellates, are applied closely to the nuclear membrane. The fact that this structure, with its capacity for growth, characterizes the Trichomonadidae, and among the polymastigote flagellates is found only in that group and its close relatives, supports the statement of Dobell that *Histomonas* and *Dientamoeba* "are both obviously related to the Trichomonadidae." The existence of a member of the trichomonad group of flagellates, *Gigantomonas herculea,* which like *Dientamoeba,* occurs in an amoeboid binucleate phase with persisting paradesmose, gives further support to that idea of affinity. Considering the reduction of mastigont structures in the amoeboid forms of *G. herculea,* one might indeed imagine the origin of *Dientamoeba* in a similar reduction of the flagellate features of a trichomonad-like form. It is clear that

the amoeboid forms of *Gigantomonas* are properly placed among the trichomonads, and would be so even if the flagellate phase had been lost; perhaps a placement of *Dientamoeba* in the flagellates, at least, is similarly justified. Just as in ciliates the infraciliature is more significant in determination of affinities than the ciliature itself, which may be greatly reduced or lost in certain species, so in flagellates such structures as the blepharoplast and paradesmose may be of systematic significance even in the absence of flagella.

## SYMBIOTES IN THE CYTOPLASM AND NUCLEUS OF GIGANTOMONAS HERCULEA

A noteworthy feature of the flagellates that inhabit termites is the frequent presence of diverse kinds of microörganisms within them. The number in the cytoplasm is especially large, when we consider the group of termite flagellates as a whole. Several sorts, however, have been found in the nucleus, especially in *Trichonympha* (Kirby, 1944*a*).

To provide an understanding of the flagellates as hosts of microörganisms, these symbiotes should be recorded and described as completely as possible, but this presents various difficulties. Most of them are available for study only on stained slides, prepared by methods adapted to the host flagellates, with no regard originally to the symbiotes. New material of the symbiotes cannot be studied, at least at present, by methods that would be appropriate to them. The life histories of the organisms can be worked out only by piecing together in probable order various specimens that are found. Consequently, not only is there uncertainty about the complete morphology and life history of the symbiote itself, but also it is extremely difficult to make comparisons with other microörganisms.

In previous papers I have directed attention to various symbiotes of termite flagellates, without following any uniform system of designation. In some papers (e.g., Kirby, 1932) they have been given descriptive epithets, based on structure and position. In others, they have been referred to by comparison with the generic names of organisms that they resemble (*"Fusiformis*-like" microörganism on Devescovininae). In still others, they have been given names of the sort customary in zoölogical nomenclature (Kirby, 1944*a*, *Caryococcus invadens*, *Caryoletira anulata*). Specific names could, of course, be assigned to all these microörganisms, as there is practically no likelihood that those which occur in flagellates of termites are specifically identical with anything already described from other hosts. Their systematic position is often uncertain, however, and generic allocation is difficult. In the frequent absence of complete knowledge of life histories, one cannot be sure whether a form in one flagellate is the same as that in another. It seems desirable, therefore, to adopt some scheme for arbitrary designation of these symbiotes. This can be done by numbers or letters better than by descriptive epithets, since the latter scheme is useful only within a particular group. The various kinds of microörganisms in *Gigantomonas herculea* can be designated by numbers. These numbers can be preceded by an abbreviation for the host

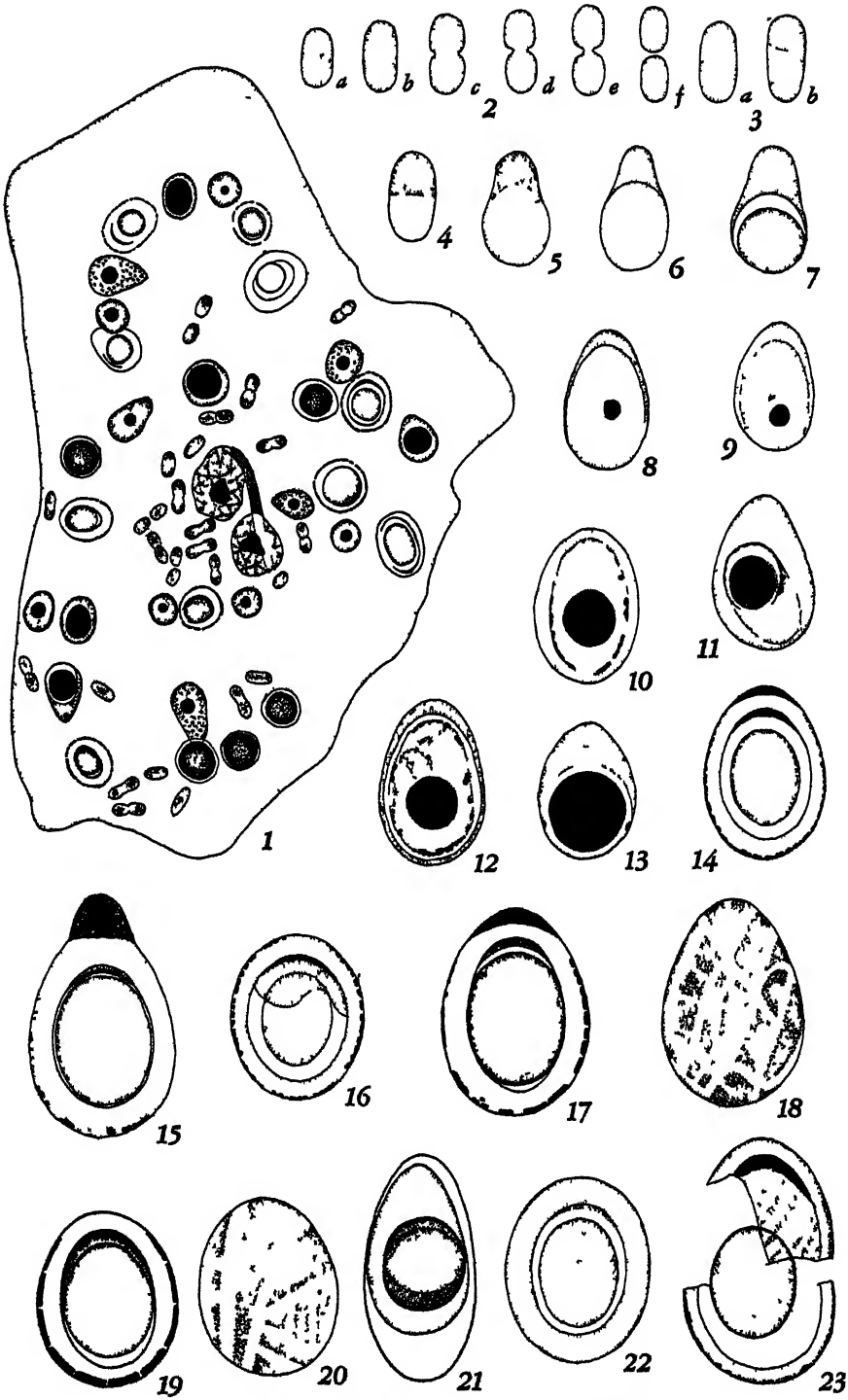


Fig. G. (For explanation of figure, see bottom of facing page.)

name, so that designation of the host in which they occur is also carried in the symbol. A few of the microörganisms can be given a complete enough diagnosis, and their systematic position can be well enough ascertained, to permit generic and specific names to be assigned appropriately. That is unquestionably true, for instance, of *Caryoletira anulata* But in general, if this treatment is ever to be accorded to most of the organisms, it should be only after as complete a study as possible is made at least of representative types, and as extensive a comparison as possible is made among the different ones and with related microorganisms. Meanwhile, the symbolic designation is sufficient to supply a means of reference, under which the facts now known can be put on record.

In this account, I will not attempt to reach any definite conclusions concerning the systematic affinities of the microörganisms described. The groups to which relationships may be conceived with reference to different microorganisms are bacteria, Chlamydozoa including Rickettsiaceae, Microsporidia, Haplosporidia, and Chytridiales. Resemblances to certain members of these groups will be considered in the accounts of the different organisms.

## Symbiote Gh1

(Fig. G, 1–23)

*Host.—Gigantomonas herculea* Dogiel in *Hodotermes mossambicus.*

T–4232. 15 mi. n. of Christiana, Transvaal. (Syntype slides TP–3067:36B, 28E.)

T–4224. 7 mi. n. of Kimberley, Cape Province. (Xenosyntype slide TP–3061:2.)

The microörganism designated as Symbiote Gh1 was found fairly abundant in sections made from several *Hodotermes mossambicus* of one colony from the Transvaal. There were some, too, in whole mounts of flagellates from the other colony recorded above. The symbiotes were found only in the large amoeboid forms of *Gigantomonas herculea* (fig. G, 1); they were sometimes so

Fig. G. Symbiote Gh1. (1. S.H. × 960. 2–23. Series of developmental stages, × 2950. 21 and 22 S.D.; others S.H.) 1. Binucleate, amoeboid form of *Gigantomonas herculea*, cytoplasm containing the symbiote in various stages of development. 2, *a–f*. Early stage of the symbiote, showing fission. 3, *a* and *b*. Cytoplasm marked off into two regions. 4. Body differentiated into two halves, one light-staining, one dark-staining. 5, 6. Light-staining part enlarged, ellipsoidal in form. 7. Ellipsoidal body separated from the remainder. 8. Ellipsoidal body containing a deep-stained granule. 9. Outer boundary of the inner body marked by a row of deep stained granules. Central granule larger than in 8. 10. Central body large and densely black. Stained masses at periphery of inner ellipsoidal region. 11. Outer shell membrane gray, homogeneous. Ellipsoidal inner body partly separated from inside of shell. 12. The deep-stained central body is large and there are numerous flecks of chromatic material in the surrounding cytoplasm. Separation from the shell distinct. 13. The dense central body has attained a maximum size, filling most of the space within the shell. 14. Optical section of fully developed spore The inner body, which formerly stained deeply, is now refractory to iron-haematoxylin. There is a caplike accumulation of stained material on the inner surface of the shell at one end, and stained material on the outer surface of the shell at the same end. 15. A protruding mass of deep-staining substance at one end of the spore. This may be the darker staining half of the earlier stage, as 5. 16. Showing the form of the cap of iron-haematoxylin-stained substance on the inner surface of the shell. 17. Optical section. Refractile inner body, cap on inner surface of shell at one end, stained bodies in shell at the periphery. 18. Surface view of same specimen as fig. 17, showing the pattern produced by the stained bodies in the outer substance of the shell. 19, 20. Optical section and surface view of another specimen, showing structures similar to 17 and 18. 21. Inner body, shell, and outer surrounding membrane which shows in Delafield-stained material. 22. The shell is stained homogeneously in Delafield's haematoxylin, showing that it is a thick, solid membrane. 23. Shell broken, enclosing the very smooth ellipsoidal inner body.

abundant as to occupy the whole cytosome, with considerable density. Various stages of development occur together in the host flagellate. In some hosts, however, the mature forms are more prevalent; in others, developmental forms prevail. There is little reason to doubt that the various forms described below actually belong in the life history of Symbiote Gh1.

The symbiotes lie in the cytoplasm individually (fig. G, 1), with no grouping of the sort that characterizes the *Sphaerita*-type of microörganism. In Delafield-stained material a narrow clear space surrounds each symbiote, or pair of symbiotes in the early fission phases, and at the outer boundary of this phase is a membrane. It is not apparent whether this membrane is the boundary of a cytoplasmic vacuole in which the symbiote lies, or is a structure belonging to the symbiote, which then is directly surrounded by cytoplasm. There are indications that the latter is the true explanation; sometimes a clear space appears on both sides of the membrane.

One form of the symbiote is an ellipsoidal body or rod with a length of 2.5 to 5$\mu$ and a width of about 1.5$\mu$, equal in size at both of the rounded ends (fig. G, 2, a, b). This is apparently an early stage in development. The rods vary in depth of staining, but all appear more or less homogeneous and in the material studied show no internal differentiation or indication of nuclei. Many of the longer rods appear to be in various stages of transverse fission (fig. G, 2, c–f).

In rod forms that evidently follow in development those described above, the size and shape is the same but iron-haematoxylin staining shows a differentiation into two parts (fig. G, 3, a–b; G, 4). The substance of one half stains less intensely than the other. Later the lighter region becomes clearly individualized and enlarged, so that one end of the rod appears swollen (fig. G, 5, 6, 7). The darker-staining part undergoes no great change in size. In later development what is apparently the same substance may still be found as an appendage or incrustation on the surface of the enlarged, ellipsoidal structure (fig. G, 15); at that time it is deeply stainable by iron-haematoxylin.

When it first begins to enlarge, this rounded structure shows no internal differentiation. A small granule that stains deeply with iron-haematoxylin then appears within it, and subsequently increases in size (fig. G, 8–13). At its periphery, set off by a space, a thick membrane develops (fig. G, 12). The membrane is often especially thick at one end; it becomes a shell-like structure, which stains solidly with Delafield's haematoxylin (fig. G, 22). That stain also reveals a thin membrane that surrounds the shell-like structure, separated by a clear space of variable width (fig. G, 21).

The nucleus-like body enlarges until it occupies almost all of the space within the membrane. In the material examined its substance was always solidly stained with Heidenhain's iron-haematoxylin. Granules that are stained by the same process were present in the cytoplasm outside of the enlarging nucleus-like body (fig. G, 10, 12); and when that cytoplasm is more or less entirely replaced in enlargement of that body, similar granules are present between it and the thick membrane. The granules are generally situated toward one end; and in specimens that appear mature they have coalesced

into a cap-shaped mass applied to the inner surface of the membrane (fig. G, 14–17). The cap-shaped mass stains intensely with Heidenhain's iron-haematoxylin, but not at all with Delafield's haematoxylin.

The inner body attains a length of about 4$\mu$, with a broad ellipsoidal form, while it still stains intensely; in what appear to be later stages it has not increased appreciably in size but is entirely refractory to iron-haematoxylin (fig. G, 14–17). At the same time, the stain reaches the cap-shaped mass through the thick membrane, indicating that it is not that membrane which excludes the stain. The outer layer of the thick membrane shows a pattern of iron-haematoxylin-staining patches of variable size and shape (fig. G, 17–20). Most often the patches are narrow and elongated. They do not show at all in Delafield-stained material.

The fully developed structure, as shown in fig. G, 14, 16, 17, 22, has a length of 8–9$\mu$, a width of 6.5–7.5$\mu$, and the membrane encloses an ellipsoidal body, refractory to stain, which measures 4.5–5 × 4–4.5$\mu$.

When Symbiote Gh1 was first seen it was thought that it might be a microsporidian (Kirby, 1941, p. 1079); but closer study has not upheld the idea of its affinity to that group of protozoa. There is no polar capsule, no polar filament, and no definite, constant nuclear structure has been found. There are points of resemblance to bacteria; and the process of development, according to the pattern described, suggests spore formation in bacteria in many particulars.

The trophic forms are bacillus-like, and they multiply by transverse fission. The region that may, in this comparison, be regarded as a spore primordium is set off from the rest of the cell by a membrane-like demarcation, and sometimes (fig. G, 7) it is set off by a clear area. Phenomena like this have been described, for instance, by Lewis (1934) in *Bacillus mycoides*. The appearance of the deep-stained granule in the apparent spore primordium, and the progressive enlargement of this body, are events comparable to what has been described in some bacilli. The figures by Petit (1921) of spore formation in a bacillus show, in fixed and stained material, a small deep-stained polar granule, which enlarges greatly and continues to be stainable, but finally does not take the stain. There are similarities, too, in what Ambrož (1909) designated as the second type of chromatin condensation in spore formation of *Bacillus nitri*. *Bacillus mycoides* shows comparable stainability changes in development of the "vorospore" (Lewis, 1934). Delaporte (1939) described how in several bacilli the spore begins in a polar chromatic granule which stains by iron-haematoxylin, and which enlarges little by little until the spore becomes mature, when it does not stain. The chromatic axial filament which Delaporte regularly found in the bacteria she studied was not seen in Symbiote Gh1. The statement by Lewis (1941) that in bacteria the spore is formed from a clear, hyaline, polar spore primordium, which is set off from the remainder of the cell by a membrane; that it results from a condensation of the substance contained in the spore primordium; and that during the early stages before the spore body has become fully condensed it stains readily, contains generalizations that can be applied to Symbiote Gh1.

The size relationships, however, are not at all comparable with what is known of bacilli. Not only do the spores become very much larger than the vegetative cells, but their actual size is relatively very great. The dimensions of about 8 × 9$\mu$ are about three times the length and five times the width of the average-sized trophic cell of Symbiote Gh1. If the pattern of development traced above is authentic, the spore obviously is not developed without very considerable addition of substance from outside; that is, it results not only from a condensation and organization of materials in the cytoplasm, but also from continued growth.

No conclusions can safely be drawn now about the systematic affinities of the Symbiote Gh1. No germination of the supposed spores has been seen, no cultural or physiological studies could be made, and the pattern presented as the course of development is based merely on an interpretation of the probable arrangement of the forms seen. If the symbiote is encountered again in its South African host, or a form similar to it is found elsewhere, further studies should be interesting and fruitful for the subject of the cytology of microörganisms.

### Symbiote Gh2

(Fig. H, 1, 2)

*Host.—Gigantomonas herculea* Dogiel from *Hodotermes mossambicus*.
T-4232. 15 mi. n. of Christiana, Transvaal. (Syntype slides TP-3067:36B, 31B, 31A.)

Like Symbiote Gh1, this microörganism was often found to be extremely abundant in the body of large, amoeboid forms of *Gigantomonas herculea*. It occurs in the cytosome in more or less spheroidal groups of varying size (fig. H, 1). The individual symbiotes are distributed evenly throughout the interior of the group, and there is no evidence of a membrane surrounding the group. A clear region is immediately around the organisms, but the boundary of this is no more than the limit of the surrounding plasma.

The individual symbiotes within the group are apparently spores, and most of them are mature. Their length is 2–2.5$\mu$, and their shape broadly ellipsoidal. In their structure they show notable resemblance to the spores of Symbiote Gh1, but they are much smaller, and consequently it is not possible to make out so much detail. There appears to be a relatively thick surrounding membrane; this shows in optical section only as a clear area bounded by an outer and an inner line, but the interpretation that is certain in Symbiote Gh1 is probably applicable to this microörganism also. The body within does not show the separation from the membrane that was observed in the other symbiote. At one end is a mass, which in optical section appears more or less crescentic, and which stains intensely with iron-haematoxylin.

I have not found a complete developmental series in Symbiote Gh2, but a number of what appear to be earlier stages occur in some of the groups of spores (fig. H, 2). The most frequent of these has a large deep-stained spherule at one end, a narrow clear zone around this, and a fairly dense cytoplasm elsewhere; it is an exact miniature of a form that occurs in Symbiote Gh1. Some other smaller forms which have been seen seem to be still earlier phases of development.

It appears likely that this microörganism is related systematically to Symbiote Gh1, but there seems to be no doubt that it is specifically different. It occurs in groups, often of a large number of spores, whereas the individuals of Gh1 are isolated in the cytosome. There is a very great difference in size, which lies far outside of the limits of variability in the forms I have seen.

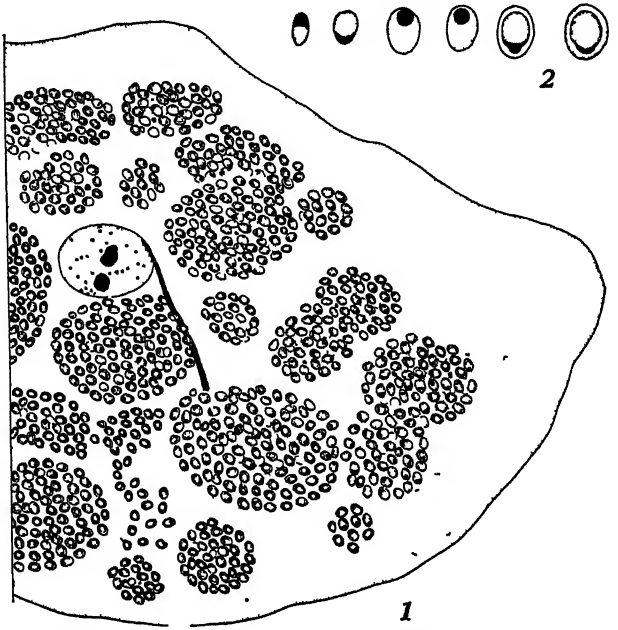


Fig. H. Symbiote Gh2. 1. Section of a binucleate, amoeboid *Gigantomonas herculea*, cytosome containing many groups of the symbiote. S.H. × 960. 2. Diagrams of individual forms of the symbiote. The figures from left to right may represent successive stages in development, which are somewhat comparable to those of symbiote Gh1, though of much smaller size. S.H. × 2950.

## Symbiote Gh3

(Fig. I, 1, 2)

*Host.*—*Gigantomonas herculea* Dogiel in *Hodotermes mossambicus*.

T-3061. Between Mbeya and Iringa, Tanganyika Territory. (Syntype slides TP-2061:7A, 7B.)

In the cytosome of large amoeboid forms of *Gigantomonas herculea* the microörganism designated Symbiote Gh3 occurs in groups that are similar in size to the larger aggregates of Symbiote Gh2. The groups are more or less spherical in shape, and are generally definitely outlined, but there is no indication of a surrounding membrane (fig. I, 1). The symbiotes appear to lie merely in a space in the cytoplasm, a great number of them together. Generally they are densely packed throughout the mass. Altogether not more than a dozen groups have been found, one or two of them being present in a single flagellate, and those were seen only on the sections listed above. On two occasions, groups were ruptured and the organisms were in part dispersed in the cytosome.

The form of the symbiote that evidently is mature, and is the only stage

present in most of the groups, has a structure which is very different from that of Symbiote Gh2. As seen from one aspect, its shape can be compared to that which characterizes the desmid *Cosmarium* (fig. I, 2). There are two halves, an equatorial constriction that is not very deep, and a relatively broad connecting isthmus. This might seem to be a fission form, but that could not be so, because all the symbiotes have the *Cosmarium* form and the depth of the equatorial constriction is constant. When turned at a right angle to the position that makes the above comparison possible, the organism has a circular outline. In the iron-haematoxylin-stained preparations the isthmus region appears clear and the two halves are usually intensely stained. The largest *Cosmarium*-shaped forms have a diameter of about 2.5$\mu$, but there are smaller ones of 1.5–2$\mu$.

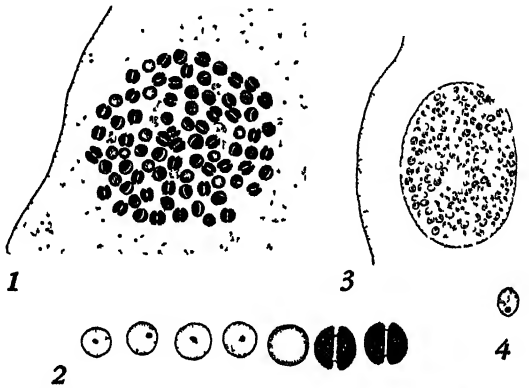


Fig. I. 1, 2. Symbiote Gh3, S.H.: 1. A group in the cytoplasm of a large amoeboid form of *Gigantomonas herculea*. × 940. 2. Individual forms of the symbiote, showing what are apparently successive stages in development. × 2875. 3, 4. Symbiote Gh4, Z.H.: 3. A group in the cytoplasm of a large amoeboid form of *Gigantomonas herculea*, surrounded by a very distinct membrane. × 960. An individual symbiote from this group. × 2950.

In a few groups there occurred, along with *Cosmarium*-shaped forms, others that are presumably earlier developmental stages (fig. I, 2). The smallest of these are about 1.5$\mu$ in diameter, are round in outline, show no equatorial constriction, and contain a relatively large deep-staining granule. The granule is central or eccentric in position. Other forms of similar or larger size have a clear equatorial band rather than a groove; subsequently a definite groove develops. The *Cosmarium*-shaped forms that then result may still show the interior nuclear granule; in later stages, as already stated, the stain is so dense that no interior structure can be observed.

### Symbiote Gh4

(Fig. I, 3, 4)

*Host.—Gigantomonas herculea* Dogiel in *Hodotermes mossambicus*.

T-4232. 15 mi. n. of Christiana, Transvaal. (Syntype slide TP-3067:24B.)

Several masses of Symbiote Gh4 were found in the cytoplasm of sectioned amoeboid forms of *Gigantomonas herculea*. Each mass is surrounded by a very distinct membrane (fig. I, 3). Throughout all the space within the membrane

the individual symbiote bodies are very numerous and closely packed. Each has a more or less spherical form, with a diameter of about 1$\mu$ or somewhat less, and contains a sharply defined black-stained granule at or near one edge (fig. I, 4). The granule is separated by a clear zone from the adjacent gray region.

### Symbiote Gh5

(Fig. J, 1–7)

*Host.—Gigantomonas herculea* Dogiel in *Hodotermes mossambicus.*

T 4017. Weenen, Natal. (Syntype slide TP-2077:3.)

T-4082. Commadagga, Cape Province. (Xenosyntype slides TP-3000:7, 12.)

T 4019. Weenen, Natal. (Xenosyntype slide TP-2075:9.)

Symbiote Gh5 has been found in both whole mounts and sections of *Gigantomonas herculea* from various colonies of *Hodotermes mossambicus* and occurs both in amoeboid forms and in flagellate forms of its host.

It occurs in the cytosome. Most often, in amoeboid forms of the host flagellate, there are numerous more or less spherical groups which are very diverse in size (fig. J, 6). Some of the groups are very small, comprising only a few individual bodies; and many of the hosts also contain isolated symbiote bodies in the cytoplasm. In the smaller, flagellate forms of *G. herculea* single, very large groups have sometimes been found.

There appears to be a membrane around each group, though it is often not very distinct. The symbiote bodies enclosed by the membrane are densely packed, as a rule, and are evenly distributed throughout the interior. In none of the groups I have seen, from the smallest to the largest, have I been able to recognize any matrix substance enclosing the individual symbiote bodies, which appear as isolated granules.

Taken all together, the spherical bodies of Symbiote Gh5 range in diameter from 0.5–2$\mu$. The smallest sizes occur in what are most likely developmental stages; but in some symbiote groups the individual bodies have a larger size than in others seemingly in the same stage of development. About 1–1.5$\mu$ is the most frequent range of diameter in mature forms.

The symbiote spherules differ in internal structure and in stainability. Some are homogeneous and have a gray iron-haematoxylin stain; others appear as rings in optical section, and stain either gray or black. Some groups have only small granules with a homogeneous appearance and a gray stain; those may be early developmental stages (fig. J, 1). In other groups in addition to similar granules are some that are larger, ringlike, and black (fig. J, 2–3). In still other groups most of the granules are of the larger size and ringlike, but they fall into two categories as regards stainability (fig. J, 4). The black rings are fewer than those that stain less intensely.

In its grouping in the cytoplasm, Symbiote Gh5 resembles organisms, occurring in the cytoplasm of many protozoa, that have been widely referred to as *Sphaerita,* a genus of the Chytridiales. In his systematic work on aquatic Phycomycetes, Sparrow (1943) gave place to certain species of *Sphaerita* and *Pseudosphaerita* in free-living flagellates and amoebae, considering mainly those chytrids seen by Dangeard. He was unable, however, to include various

other organisms which had been described under the name *Sphaerita,* because not enough data were provided for systematic treatment in his account. In my review (1941) of organisms living in the bodies of protozoa, I followed the

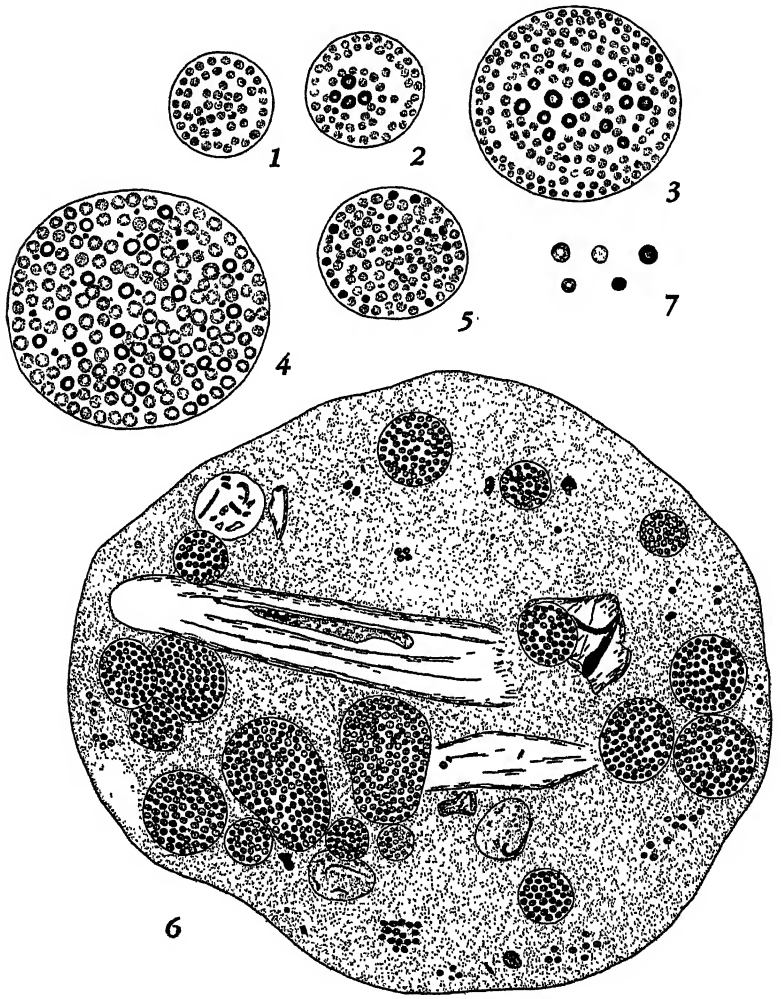


Fig. J. Symbiote Gh5. (1–3. Groups from a single binucleate amoeboid host. S.H., × 1880.) 1. The smallest groups show only gray spherules. 2. In most of the groups there are deep-staining rings and granules in the interior. 3. The latter are more numerous in the larger groups. 4–5. Two groups from another amoeboid host: The individual symbiote bodies in the smaller group are much smaller in size than are those in the larger group. The bodies in the larger group, especially the stained ones, appear as rings; in the smaller group there are fewer deep-stained bodies, and they appear solid. S.H. × 1880. 6. An amoeboid binucleate *Gigantomonas herculea* with many groups of Symbiote Gh5, in various sizes, in the cytosome. S.H. × 960. 7. Detail of symbiote forms from same specimen. × 2950.

custom of protozoölogists and treated all these symbiotes as belonging in the Chytridiales. It must be admitted, however, that in the absence of observations on living material, concerning among other things the formation, behavior, and flagellation of zoöspores, such an assignment is questionable, as

Sparrow recognized. In many organisms we do not even know that a multinucleate thallus is formed; the spherules that are seen may perhaps be isolated at all times. Studies of fixed and stained material should, if complete, be more adequate morphologically than those based only on living material; but none of the accounts of so-called *Sphaerita* in endozoic protozoa have provided indisputable evidence of the presence of the features necessary for placement in that genus. For decision on the position of symbiotes such as Gh5, studies of the complete life history are needed.

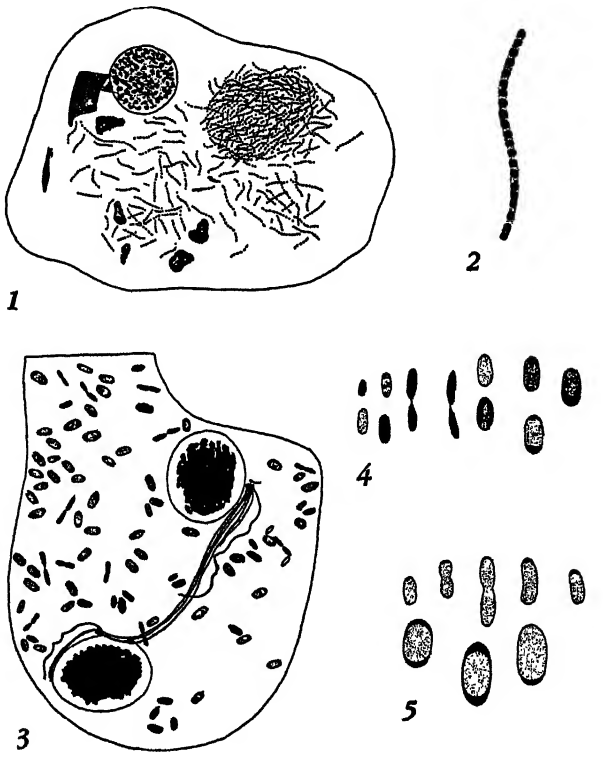


Fig. K. (1 and 2. Symbiote Gh6. 3–5. Symbiote Gh7. S.H.) 1. Section of *Gigantomonas herculea*, uninucleate form with part of cresta. Moniliform filaments in cytoplasm. × 960. 2. Freehand diagram of one of the filaments from the specimen represented in fig. K, 2. 3. Part of the body of an amoeboid *Gigantomonas herculea* with paradesmose and small new crestas. Symbiote scattered in cytoplasm. × 900. 4. Forms of the symbiote from the specimen of fig. K, 3. Stout rods, fission forms, and bodies with deep-stained regions at one or both ends. × 2200. 5. Forms of the symbiote from another specimen. Stout rods and fission forms. The ellipsoidal, apparently mature forms show well-defined caplike-stained substance at the ends. × 2200.

## Symbiote Gh6

(Fig. K, 1–2)

*Host.*—*Gigantomonas herculea* Dogiel in *Hodotermes mossambicus*.

T-4232. 15 mi. n. of Christiana, Transvaal. (Syntype slide TP-3067:36B.)

The symbiote designated Gh6 was seen in only a few specimens on this slide. That it is an organism actually living and growing in the cytoplasm is sug-

gested by its distribution and appearance. There are many moniliform filaments, which range in length from 2–3$\mu$, or less, to 8–10$\mu$. Each filament is made up of a row of deep-stained granules, evenly spaced and close together (fig. K, 2). It cannot be said whether or not the granules are separate, but connected, bodies; they may be in a matrix of nonstaining, continuous substance. The size of the granules is about the same in all the filaments; differences in length are correlated with proportionate differences in the number of granules. Filaments are dispersed throughout the cytoplasm, and a large number of them are aggregated in a large mass in the specimen represented (fig. K, 1). In the mass the filaments are dense and form a rounded group, but the group is not enclosed in a vacuole. Around the edges filaments extend out freely into the surrounding cytoplasm.

Symbiote Gh6 has some morphological resemblance to the symbiote which occurs in all specimens of *Trichonympha campanula* and *T. collaris*, and which was described by me (1932) under the name "proximo-nuclear parasite." Its form further suggests that of rickettsiae in certain phases. In some features of structure and also in size there is, for instance, a certain similarity to the thread forms of *Rickettsia prowazeki* and *Dermacentroxenus rickettsi* found by Pinkerton and Hass (1932, *a* and *b*) in the cytoplasm of tissue-culture cells. Such forms were shown also by Wolbach (1919). But consideration of these morphological similarities is of no great value in defining the systematic position of the organism.

### Symbiote Gh7

(Fig. K, 3–5)

*Host.—Gigantomonas herculea* Dogiel in *Hodotermes mossambicus*.
T-4019. Weenen, Natal. (Syntype slides TP-2075:13, 9.)

Symbiote Gh7 is fairly frequent on a series of slides made from termites at Weenen. It is scattered in the cytoplasm, in no particular grouping (fig. K, 3), and may be present in great abundance in its hosts. The larger forms of the symbiote are rods about $1\mu \times 3\mu$, with rounded ends (fig. K, 5). Smaller forms grade down to $0.5\mu \times 2\mu$ or less, and frequently occur in binary fission (fig. K, 4).

The interior structure of the symbiote is generally not very well defined in the material I was able to study. The cytosome showed stained and nonstained areas, but no body was seen that could be regarded as a nucleus. In some specimens the bacilliform, elongate symbiotes show deep-stained caplike regions at the two ends (fig. K, 5).

Descriptions of forms similar in shape and caplike stained ends can be found in the literature concerning a variety of subjects. For instance, there are diagrams suggesting some forms of Symbiote Gh7 in figures of Lindner bodies reported in trachoma (Heymann and Rohrschneider, 1930, fig. 7); in figures of the supposed spores of *Cytorycles variolae*, thought by Calkins (1904) to be a sporozoan associated with smallpox; in figures of the supposed spores of *Caryoryctes cytoryctoides*, described by Calkins (1904) in the macronucleus of *Paramecium;* and in drawings of the ring-shaped bodies of *"Theileria tsutsugamushi,"* reported by Hayashi (1920) in the blood cells and plasma

of patients suffering from tsutsugamushi disease. The form is not, evidently, characteristic of a particular kind of organism. It is a type of body which may occur in various microörganisms, and also in certain cell inclusions.

## Symbiote Gh8

(Fig. L, 1, 2)

*Host.*—*Gigantomonas herculea* Dogiel in *Hodotermes mossambicus*.
T-4017. Weenen, Natal. (Syntype slide TP-2077:4.)

Symbiote Gh8 was seen only in amoeboid, binucleate specimens of its host. A group in a large vacuole is surrounded by a relatively thick membrane.

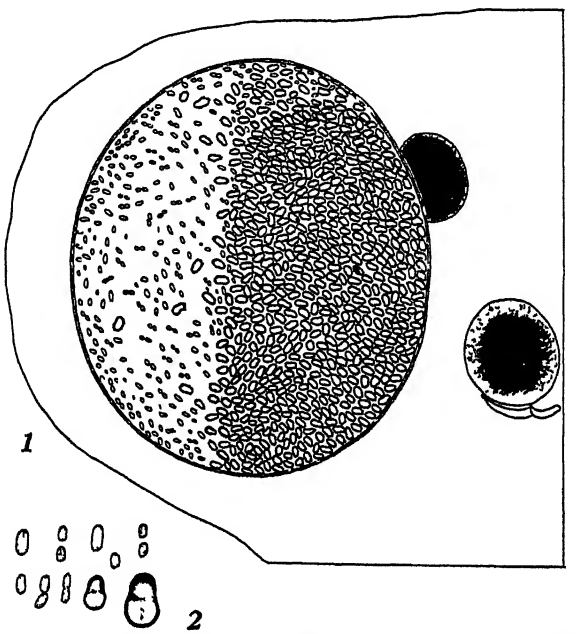


Fig. L. Symbiote Gh8. 1. Part of the body of a binucleate, amoeboid *Gigantomonas herculea*. A large vacuole, with a distinct membrane, containing the symbiote, mostly massed in one part. At the periphery in the other part are small forms, many in fission, that probably are early developmental forms of the symbiote. S.H. × 675. 2. Small rods, fission forms, and the larger, shallowly constricted bodies with deep-staining margin that make up the dense mass in fig. 1. × 2200.

What seem to be the mature forms of the symbiote have a length of 2–3$\mu$, a stout elongate form, and are typically smaller in diameter at one end than at the other. The body margins are often shallowly constricted near the middle. Around the periphery is a layer of substance that stains deeply with iron-haematoxylin. These forms were very densely packed in one half of the vacuole, and there were few in the remainder. In the region where they were sparse, numerous much smaller forms were visible at the periphery of the vacuole. These were like rods in form, with a length ranging from less than a micron to about 1.5$\mu$, and many showed binary fission. Though intermediate stages

in complete series of development to the larger forms were not found, these symbiotes probably represent early stages and later stages of the same microorganism.

### Symbiote Gh9

(Fig. M, 1–3; pl. 4, fig. 12)

*Host*—*Gigantomonas herculea* Dogiel in *Hodotermes mossambicus*.

T-4019. Weenen, Natal, South Africa. (Syntype slides TP-2075:9, 11.)

T-3061. Between Mbeya and Iringa, Tanganyika Territory. (Xenosyntype slides TP-2061: 2C, 7B, 9A.)

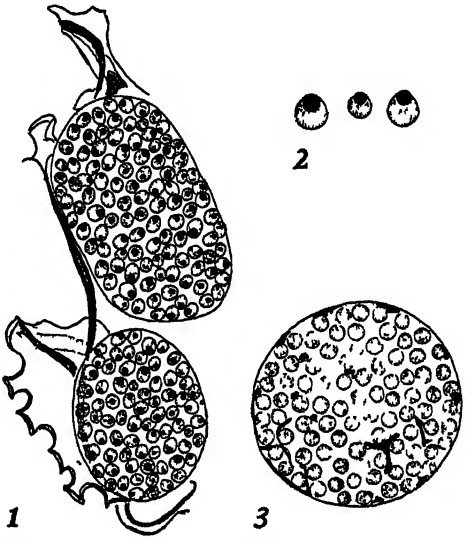


Fig. M. Symbiote Gh9. S.H. 1. Nuclei of a division form of *Gigantomonas herculea*, connected by a paradesmose, with two large new crestas, substance of both nuclei completely destroyed and replaced by symbiote. The nuclei are greatly enlarged. × 960. 2. Individual symbiote bodies from another, also greatly enlarged nucleus. × 2200. 3. Parasitized nucleus, chromatin reduced to mainly peripheral flecks. The nucleus is not enlarged and the symbiote is present in the form of homogeneous spherules, possibly in a matrix. This probably is symbiote Gh9 in an early stage of development. × 2200.

Symbiote Gh9 occurs in the nucleus, and has been found in both flagellated and amoeboid phases of its host in termites from several localities in East Africa and South Africa. Dogiel's figure 26 possibly represents the same organism; his idea of the significance of the figure is, however, very different from mine. The nucleus he represented is much enlarged and is filled with large granules; he thought that it represented the inception of nuclear division in which the interior of the nucleus becomes filled up with a great number (over a hundred) of rather large, round chromatin granules—the chromosomes. These chromosomes, he thought, later elongate and assume the shape of threads.

When the parasite is fully developed, the nucleus has become much enlarged and the chromatin and nucleoli have disappeared. In stages in which the normal nucleus has a longer diameter not exceeding 16$\mu$, it may be enlarged to as much as 33$\mu \times 20\mu$, which is the size of the larger of the two nuclei represented in plate 20, figure 12.

In some of the parasitized nuclei, as in the one represented in figure M, 1, a connecting paradesmose is present and large new crestas have developed. It is unlikely that mitotic division of parasitized nuclei would take place, so parasitization probably had occurred after division had been completed. The nuclei evidently remain in this stage with connecting paradesmose for some time.

The nuclei are filled with a closely packed mass of spores, which are about 1.5–2.5$\mu$ in diameter, and broadly ellipsoidal in form. When differentiation has been sufficient for internal structure to be made out, a relatively large granule appears at one end (fig. M, 2). The rest of the spore contents takes a gray stain with iron-haematoxylin. Sometimes the whole spore is stained densely, as was evidently true in those represented by Dogiel.

Some nuclei that are enlarged little or not at all contain what is probably the same parasite in an earlier stage of development (fig. M, 3). Homogeneous spherules fill the interior; these generally stain gray in iron-haematoxylin preparations. Vestiges of chromatin are present, in the form of flecks and filaments distributed mainly at the periphery.

In its occurrence in the nucleus, the effect on the nucleus, and the shape and arrangement of the spores, Symbiote Gh9 suggests *Nucleophaga*. It resembles the microörganism in the nucleus of *Caduceia theobromae* shown by me (1941, fig. 218, L) and referred to as *Nucleophaga,* and that in the nucleus of *Trichonympha chattoni* described by me (1944*a*, pl. 15, fig. 56) and designated a *Nucleophaga*-like parasite. Sparrow (1943) considered *Nucleophaga* to be a genus of uncertain relationships; the generic identity of various intranuclear parasites of protozoa, which have been described under that name, with *Nucleophaga amoebae* Dangeard has obviously not been established. Other intranuclear organisms occur in *Trichonympha* (Kirby, 1944*a*), as well as in higher animals. Symbiote Gh9 can be designated *Nucleophaga*-like, perhaps; but decision about its systematic position must await further study of it and similar microörganisms.

## SUMMARY

In *Hodotermes mossambicus* in East Africa and South Africa, and in *Microhodotermes viator* in South Africa, *Gigantomonas herculea* Dogiel, 1916 has been studied. *Myxomonas polymorpha* Dogiel, 1916 is a synonym of *G. herculea;* it consists of certain phases in the life history of that flagellate.

The new subfamily Gigantomonadinae, which is a group of trichomonad flagellates and is closely related to the Devescovininae, is established for *Gigantomonas* as the type genus. It corresponds to the family Amoebomitidae of Dogiel, 1922; that family name is not valid.

*Gigantomonas herculea* has a flagellate form and amoeboid forms. The former has three anterior flagella, a trailing flagellum, a stout axostyle, and a large cresta. This form is similar in structure to members of the subfamily Devescovininae, except for absence of the parabasal body.

Amoeboid forms, which show amoeboid activity and attain a much larger size than the typical flagellate form, have a prominent place in the life history. Division takes place only in these forms.

The division series that most resembles that characteristic of trichomonad flagellates results in typical flagellate forms. Morphogenetic processes involve the complete reorganization of all extranuclear organelles.

A common form of *Gigantomonas herculea* is a relatively large amoeboid type with two nuclei and persisting paradesmose. The paradesmose eventually disappears and cytoplasmic division separates the nuclei. These forms show variability in development of the mastigont structures.

The binucleate form has an organization which suggests that of *Dientamoeba fragilis* in the amoeboid body, in the two nuclei, and in the persisting paradesmose. A discussion is given of the significance of this resemblance in regard to the possible flagellate affinities of *D. fragilis*.

Nine kinds of symbiotes have been found in *Gigantomonas herculea*, one in the nucleus and the others in the cytoplasm.

Symbiote Gh1 is bacillus-like, and it develops a relatively large sporelike body by a process which suggests spore formation in bacteria. Early in the development of the apparent spore primordium, a small iron-haematoxylin-staining granule appears in it; the granule gradually enlarges to a deep-staining body of the full size of the ellipsoidal body that contained it. The ellipsoidal body is, in its final condition, refractory to stain.

Symbiote Gh5 has features that correspond to those of certain parasites of protozoa that have been considered to be *Sphaerita*. It is pointed out, however, that, in this and many other *Sphaerita*-like symbiotes in protozoa, not enough is known to permit being positive about the systematic position.

The intranuclear symbiote, Gh8, resembles forms that have been regarded as *Nucleophaga;* likewise, the true affinity of the organism is uncertain.

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# PLATES

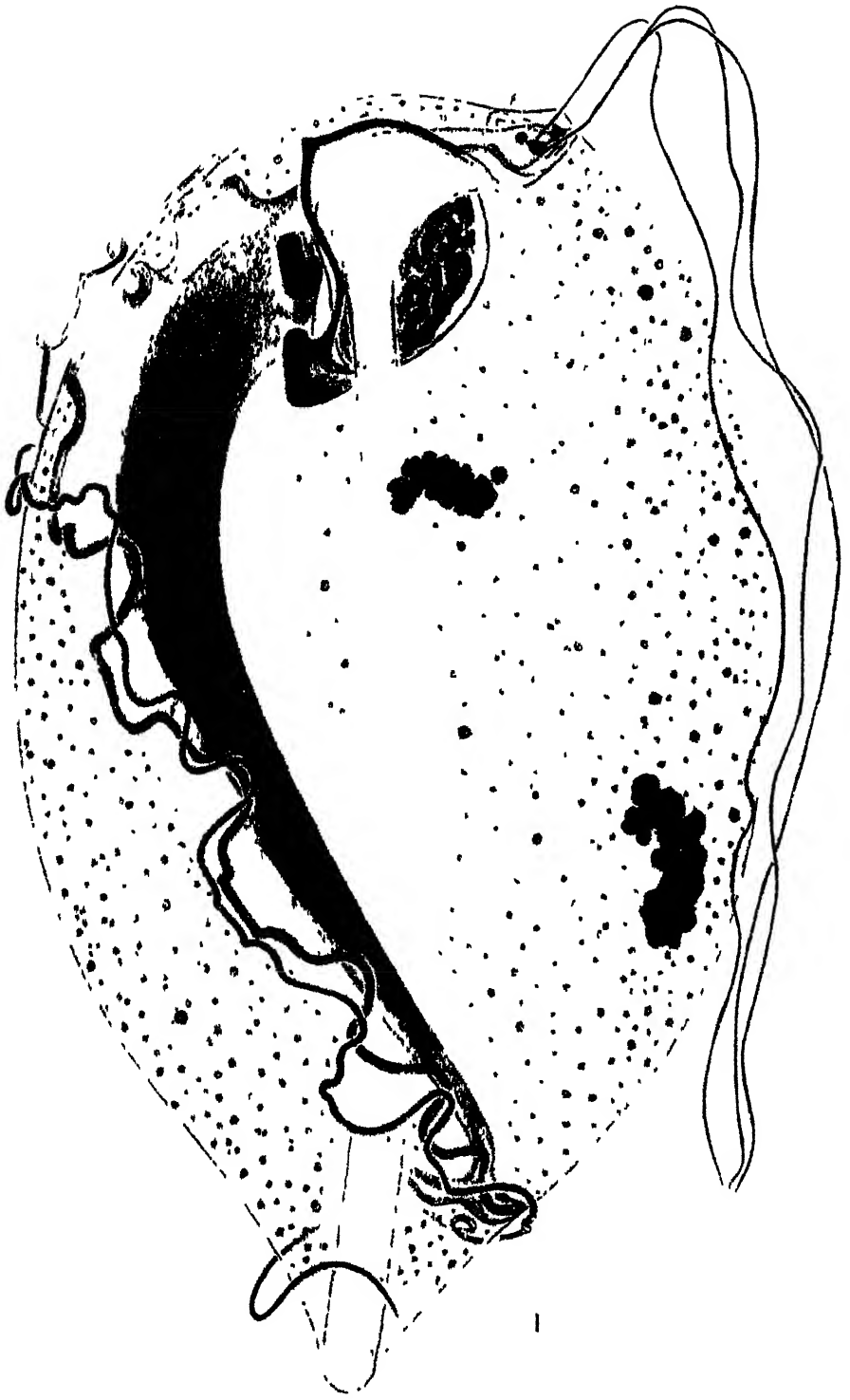
All figures have been drawn with the aid of the camera lucida.

Abbreviations for methods of preparation: H., Heidenhain's iron-haematoxylin; S., Schaudinn's fluid; Fl., Flemming's fluid; R., Regaud's haematoxylin; Z., Zirkle's copper-dichromate fixative.

## PLATE 17

*Gigantomonas herculea* from *Rodotermes mossambicus*.

Fig. 1. The specimen is normal in form and is typical of the flagellated phase of *Gigantomonas herculea*, except that in it the axostyle is blunt instead of pointed posteriorly and there is a finger-like appendage on the anterior part of the cresta. In most specimens the trunk of the axostyle is like that of figure 2, plate 18. The cresta is imbedded in the cytoplasm with a clear zone adjacent to it. Its undulated outer edge is at the surface of the body. The trailing flagellum parallels this edge, and continues in a short free flagellum. The inner part of the cresta is flat and deep-staining. At the anterior right of the cytosome are two granules, near which three long anterior flagella extend free from the body. In its anterior part, the axostyle is somewhat expanded in a capitulum on the right side of the nucleus. A narrowed prolongation of this extends to the region of the blepharoplasts. The dorsal border of the capitulum as seen here appears as a deep-stained fibril, and the membrane is prolonged posteriorly in an armlike appendage that reaches around the posterior end of the nucleus. A deep-stained, short, anteriorly directed appendage here originates from the anteromedial border of the cresta; it is not present in most specimens. Length 59$\mu$, width 36$\mu$. S.H. × 3000.



## PLATE 18

*Gigantomonas herculea* from *Hodotermes mossambicus*.

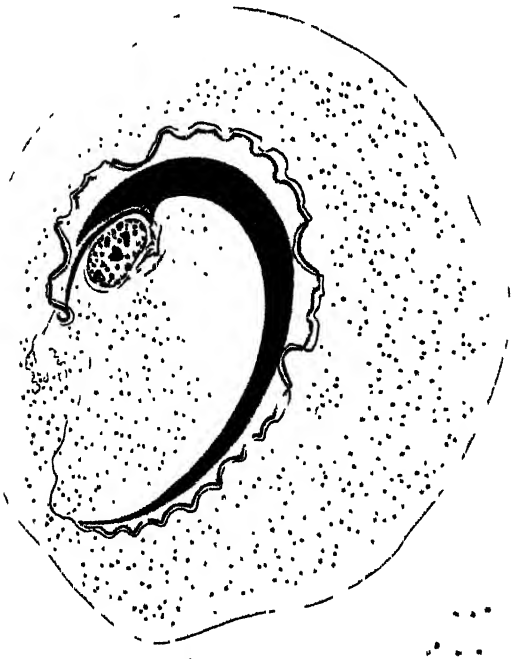
Fig. 2. The trailing flagellum more or less parallels the undulated outer margin of the cresta, but is not attached to it in most places. The axostyle is pointed posteriorly, entirely enclosed in the cytoplasm, and somewhat expanded in a capitulum alongside the nucleus. Except for the presence of the 3 anterior flagella and 1 trailing flagellum, this specimen resembles what Dogiel called type A of *Myxomonas polymorpha*. (Cf. fig. A, 3.) S.H. × 1173.

Fig. 3. A portion of a partly isolated mastigont, the body having been ruptured in preparation. Axostyle, cresta, and nucleus are bound up in a union, with no alteration of their relative positions. Three anterior flagella arise in two roots, have a length of 80$\mu$. Near their origin is a large granule; the flagella meet a pair of smaller granules near this. Another small granule is posterior to these granules. An S-shaped filament (nuclear rhizoplast) extends posteriorly around the large granule to meet the nuclear membrane. The trunk of the axostyle is distinct; posteriorly it is enclosed in the cytoplasm and pointed. S.H. × 2333.

Fig. 4. Outline of nucleus. Anterior part of cresta, showing the anteromedial edge adjacent to the nuclear membrane and extended anteriorly. Origin of three anterior flagella. The axostyle and its capitulum are not shown. S.H. × 2400.

Fig. 5. A specimen in what appears to be essentially unaltered form. The transverse position of the cresta, instead of the longitudinal position represented in figures 1 and 2, is normal in some specimens. The core of the axostyle is deep-stained, corresponding to what Dogiel considered characteristic of type A of *Myxomonas polymorpha*. The trailing flagellum is shown along the cresta margin. Anterior flagella are present, but cannot be clearly traced except at their origin; they are omitted from the figure. Length of body 72$\mu$, width 44$\mu$, nucleus 9$\mu$ × 8$\mu$. S.H. × 1270.

Fig. 6. The cresta and trailing flagellum are buried in the cytoplasm, and there is no indication of the appendage of the inner border of the cresta that appears in fig. 1. The capitular membrane is shown, expanded on the right side of the nucleus and elevated in a ridge which continues in an appendage around the posterior end of the nucleus. Two granules at the base of the flagella, the left one longer and somewhat quadrangular, the other smaller and giving rise to the anterior flagella. S.H. × 2400.



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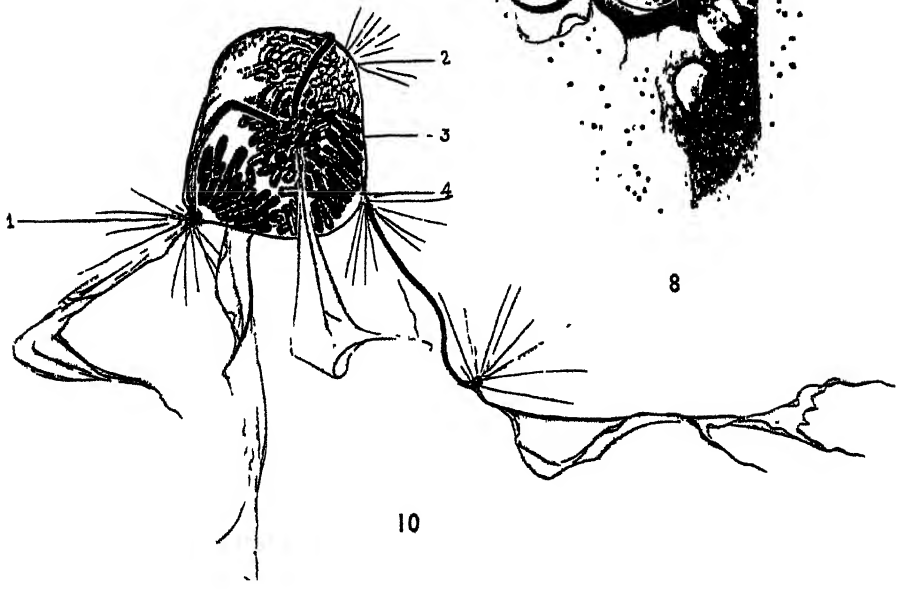


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## PLATE 20

*Gigantomonas hirculea* from *Hodotermes mossambicus*.

Fig. 11. Modified cytosome with a thick, gray outer zone, an inner granular region, and a clear zone between these. This form suggests a cyst, and appears to be comparable to what has been reported as encystment in some trichomonad flagellates. Actually this is a degenerative form that has nothing to do with encystation. S.H. × 935.

Fig. 12. Two nuclei connected by a paradesmose. Both nuclei are parasitized by the intranuclear Symbiote Gh9 (cf. p. 206, fig. M) and are greatly enlarged. S.H. × 670.

Fig. 13. Late division in an elongate amoeboid body, 150$\mu$ long. The new crestas are large and deep in the cytoplasm, and new axostyles have developed. The paradesmose is a very long strand, 120$\mu$, connecting the two mastigonts. Fl.R. × 770.

Fig. 14. A dimastigont specimen resulting from delayed division. Each mastigont is completely reorganized, with anterior flagella and trailing flagellum, fully developed crestas and axostyles. There is no trace of a paradesmose. S.H. × 1175.

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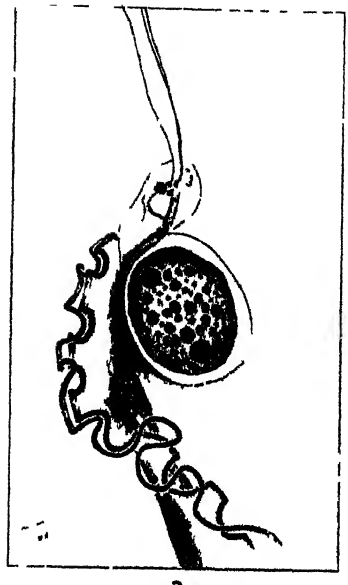

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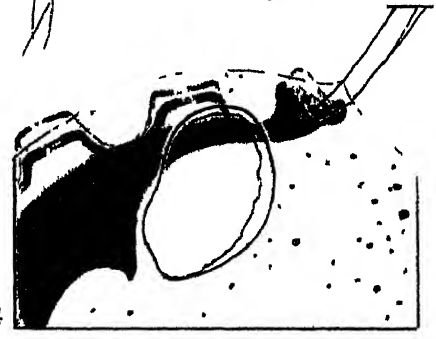

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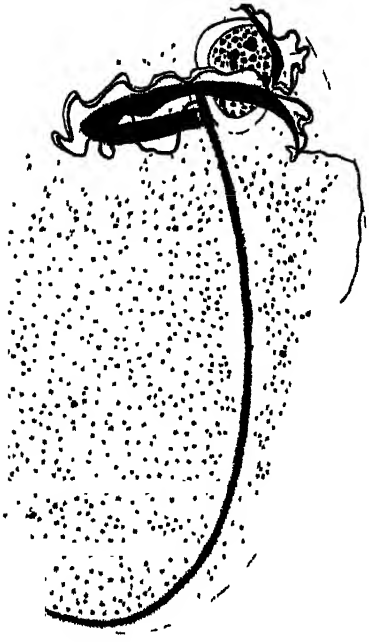

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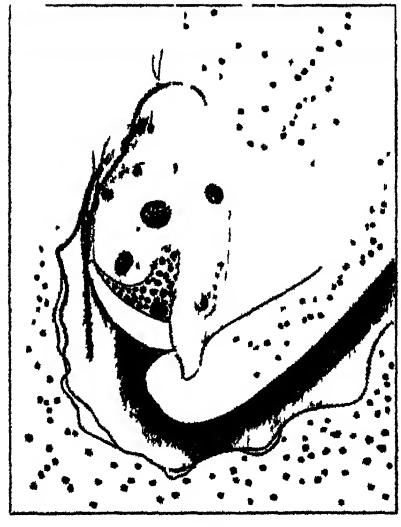

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## PLATE 19

*Gigantomonas herculea* from *Hodotermes mossambicus*.

Fig. 7. The cresta lies flat and is well represented; its outermost margin is deep-stained. Outside of this, separated by a narrow clear space, is the trailing flagellum. The whole cresta and the flagellum are deeply embedded in the cytoplasm and the free terminal part of the flagellum is also enclosed. The three anterior flagella, whose full length is not clear, are enclosed in the cytoplasm. Dogiel's type A of *Myxomonas polymorpha* is represented by specimens like this, in which the flagella were not seen. S.H. × 2400.

Fig. 8. The cresta consists of a deep-stained inner part and a clearer undulated outer part, and it may be flat as in figure 7 or curved as in this figure. A stained, irregularly formed, flattened structure is present in the cytoplasm. S.H. × 1770.

Fig. 9. Anaphase of nuclear division. The paradesmose is fibrillar, the fibrils ending in deep-stained knoblike structures at the poles. Interzonal granules lie between the two groups of chromosomes. The new crestas are filaments. Fl.R. × 2335.

Fig. 10. Multipolar division figure. Four groups of chromosomes, each in the usual anaphase arrangement, are enclosed in the nuclear membrane. Each group has as many chromosomes as in the usual anaphase. The four poles are numbered. Poles 1, 2, and 3 are connected by 3 paradesmoses arranged in a triangle. At pole 4, the end of a paradesmose is also present, but the paradesmose extends out into the cytoplasm, and its other end is free. Around each of poles 1, 2, 3, and 4 there is a single cluster of astral rays; and one is present also around the free end of the free paradesmose. Four new crestas are attached to poles 1, 3, and 4, and to the free end of the free paradesmose. This end apparently may have been detached from pole 2, but it has its own enlargement and aster, and the body of the specimen has not suffered evident mechanical damage. Fl.R. × 1175.

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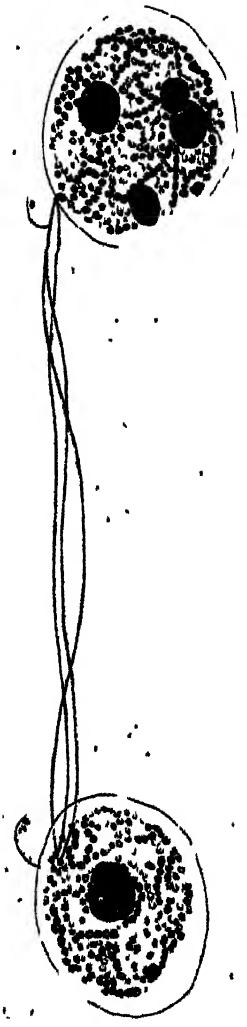

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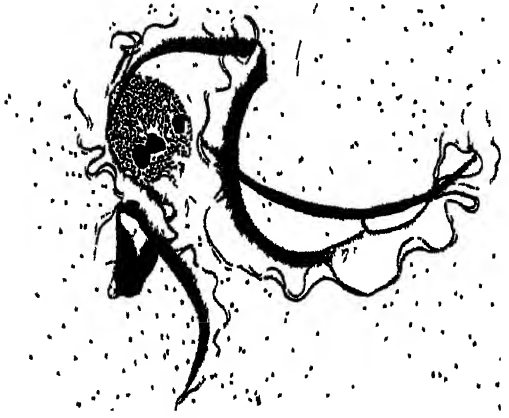

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## PLATE 22

*Gigantomonas herculea* from *Hodotermes mossambicus*

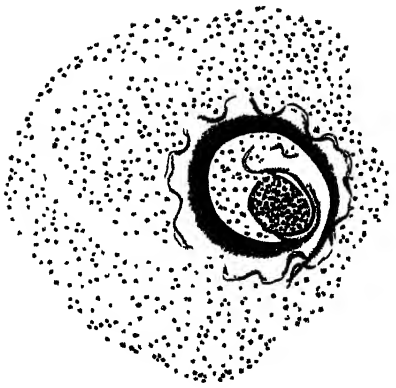
Fig. 18. Mastigonts from a large amoeboid form. Each possesses a capitulum and slender axostyle trunk, and three long anterior flagella, but no new cresta. The fibrillar paradesmose is twisted in its middle part. S.H. × 1150.

Fig. 19. Detail of one of the nuclei of the pair represented by fig. F, 4. The body is a large amoeboid, about 250$\mu$ × 125$\mu$, and it contains two pairs of nuclei. In this figure the new cresta and the paradesmose have been omitted, but one large deep staining granule at the end of the paradesmose is shown, and adjacent to it is the anterior part of the capitulum of the axostyle. S.H. × 2340.

Fig. 20. A stage similar to the form from which fig. 19 was taken, with moderate-sized new crestas; a slender axostyle trunk, not seen in the other specimen, is present. S.H. × 1590.

Fig. 21. Pair of nuclei from a large amoeboid body, nuclei connected by the paradesmose. Extending from the pole along each nuclear membrane is a deep-stained strand that is probably the new cresta. S.H. × 1056.

Fig. 22. The nuclei are close together and the fibrillar paradesmose is curved in a loop. Four fibrils are distinct. A deep-stained band-shaped strand, shorter than the nuclear diameter, connects to each pole and is applied to the membrane of each nucleus. This is probably the new cresta. S.H. × 2340.



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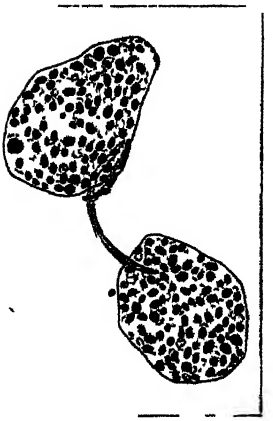

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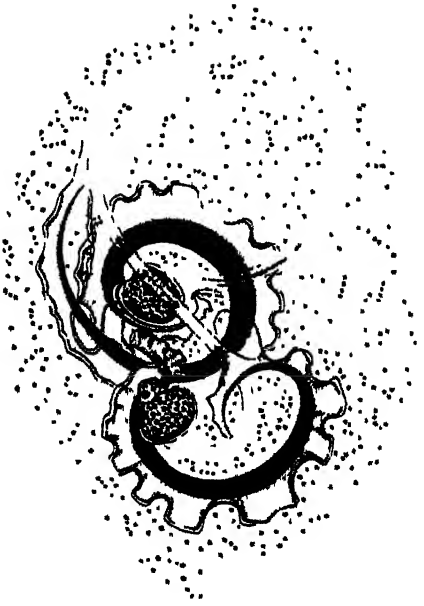

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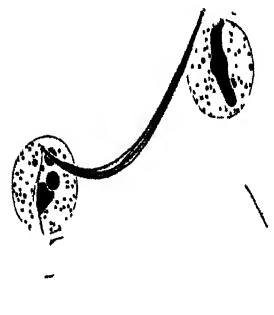
## PLATE 21

*Gigantomonas herculea* from *Hodotermes mossambicus*

Fig. 15. A normal posttelophase, in an amoeboid body 130$\mu$ × 70$\mu$. Diameter of nuclei 12$\mu$. The mastigonts are fully reorganized, crestas and trailing flagella deeply embedded in cytosome, anterior flagella enclosed in cytosome for much of their length. The two mastigonts are connected by a paradesmose. S.H. × 1250.

Fig. 16. A specimen in the same stage of development as fig. 15, amoeboid body 200$\mu$ × 60$\mu$. The body is broken only a little at the edge, and otherwise appears not to have suffered appreciable mechanical injury. One mastigont, complete for all extranuclear structures, has no nucleus. S.H. × 1200.

Fig. 17. Two nuclei 15$\mu$ in diameter, connected by a paradesmose 44$\mu$ long, in a large amoeboid body. The paradesmose consists of three separate, independently twisted strands; at the poles are two granules at one end, only one showing at the other. There is no indication of old or new crestas, and the axostyle is represented only by a portion of the capitulum that appears in the region of the poles of the paradesmose. One of these nuclei is represented in detail by fig. F, 5. S.H. × 2180.



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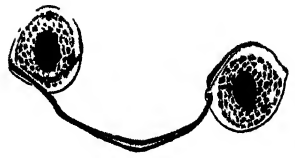

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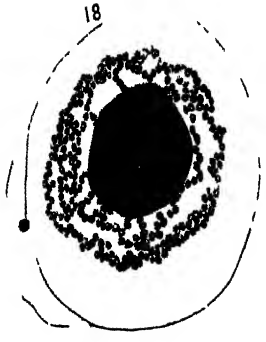

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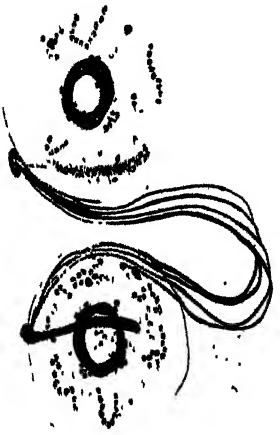

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## PLATE 23

*Gigantomonas herculea.* Figs. 23–25 from *Hodotermes mossambicus;* figs. 26–27 from *Microhodotermes viator.*

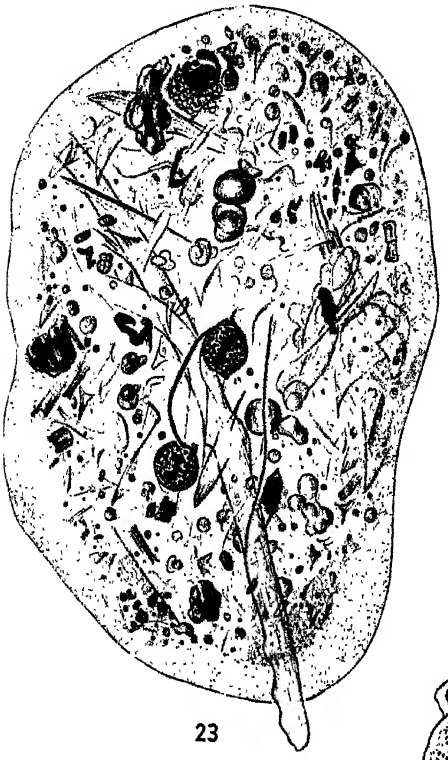
Fig. 23. A typical large amoeboid form of the flagellate. The cytoplasm contains a large number of inclusions, mostly fragments of plant material. There are two nuclei, connected by a paradesmose. There are no new axostyles or new crestas. This form was considered by Dogiel to be type D of *Myxomonas polymorpha.* (Cf. fig. A, 6.) S.H. × 636.

Fig. 24. An amoeboid form 130$\mu$ × 80$\mu$. There are two nuclei but no trace of a paradesmose, and no extranuclear mastigont structures can be seen. This specimen corresponds to Dogiel's text figure 3, considered to represent type E of *Myxomonas polymorpha.* (Cf. fig. A, 8.) S.H. × 690.

Fig. 25. An amoeboid form about 56$\mu$ × 72$\mu$, with one nucleus. There are two basal granules on the nuclear membrane, and extending from one of them a strand that curves against the nuclear membrane. This strand is probably the beginning of a new cresta. This kind of organism is Dogiel's type C of *Myxomonas polymorpha.* (Cf. fig. A, 5.) S.H. × 1150.

Fig. 26. A very large amoeboid form with 36 nuclei, each with a diameter of about 8$\mu$. There are no paradesmoses, no crestas, no axostyle trunks, and no flagella. The flagellate affinities of the organism are shown by the pair of granules on or near each nuclear membrane, and the capitulum membrane associated with each nucleus. S.H. × 405.

Fig. 27. One nucleus from the specimen of fig. 27, with the granules and capitulum membrane. S.H. × 2340.



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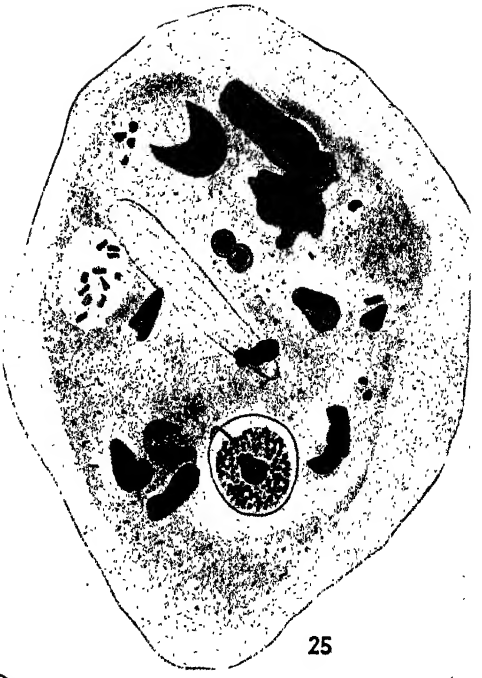

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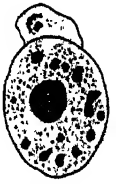

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# THE CHARACTERISTICS OF THE FLAGELLATE MONOCERCOMONAS VERRENS SP. N., FROM TAPIRUS MALAYANUS

BY

BRONISLAW HONIGBERG

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOÖLOGY
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# ERRATA

Page 230, ¶ 3, line 3, read : averaging $7.1 \times 5.1\mu$.

Page 232, legend, figure C, ¶ 1, line 11, read : (to the observer's left)

Page 232, legend, figure C, ¶ 2, line 3, read : v.p. = ventral extension of pelta.

Page 235, Summary, ¶ 3, lines 1 and 2, read : averages $7.1 \times 5.1\mu$.

Honigberg, "The Characteristics of the Flagellate Monocercomonas Verrens Sp. N., From Tapirus Malayanus," *Univ. Calif. Publ. Zoology.* Vol. 53, No. 5, 1947

# THE CHARACTERISTICS OF THE FLAGELLATE MONOCERCOMONAS VERRENS SP. N., FROM TAPIRUS MALAYANUS

BY

BRONISLAW HONIGBERG

## INTRODUCTION

IN THE SUMMER of 1945 I had an opportunity to examine fecal material from *Tapirus malayanus* in the San Francisco Zoölogical Gardens. Among other protozoan parasites I found a flagellate belonging to the genus *Monocercomonas* Grassi, 1879.

Da Fonseca (1940) found a new species of *Eutrichomastix* (= *Monocercomonas*) in feces of *Tapirus raulinus* and named it *E. bertholdoi*. When the host-parasite relationships in the genus *Monocercomonas* are considered, it is apparent that in most instances the same species of flagellate may occur in closely related hosts. This situation seems to hold true in many instances where the phylogenetic relationship is far more distant than that between *Tapirus malayanus* and *T. raulinus*. Thus it might be expected that the flagellate described by Da Fonseca and that found by me would belong to the same species. In order to establish the relationship between the two organisms there was a need of material which would provide a fair basis for comparison. In spite of the fact that the same techniques for fixation and staining were used by Da Fonseca and by me, sufficient evidence was lacking to establish the identity of the two flagellates. Although the measurements closely correspond to those reported by him, the description and illustration (fig. 7 by Da Fonseca, 1940) differed considerably from the present findings. It thus became apparent that only by examining the material used by Da Fonseca would it be possible to determine whether we both dealt with the same organism. Since it was not feasible to secure that material, I have assigned a new specific name *Monocercomonas verrens* to the flagellate from *Tapirus malayanus*.

In the present paper the nomenclature laid down by Travis (1932) was followed. Thus the name *Eutrichomastix* Kofoid and Swezy, 1915, which has been customarily applied to the genus under consideration, has been abandoned and, on the basis of priority, the name *Monocercomonas* Grassi, 1879 is used.

I wish to express my gratitude to Professor Harold Kirby, who stimulated this research and whose advice and criticism led to its completion. Credit is also due to Mr. Carey Baldwin, Director of the San Francisco Zoölogical Gardens, whose wholehearted coöperation made this project possible.

## MATERIAL AND METHODS

In the present investigation both fecal and culture material were studied in darkfield as well as in fixed and stained preparations.

The flagellates could be maintained on modified Boeck-Drbohlav (Ringer-egg-slant-serum) medium. The cultures were kept at 37° C. and transferred every 72 hours.

Both fecal smears and culture material were fixed in Hollande's cupric picroformol and in Schaudinn's fluid. The former fixative was used for preparations to be impregnated in protein silver, and the latter for those to be stained in iron hematein.

The procedure described by Bodian (1936) and Cole and Day (1940) and adapted by Kirby (1945) was used for the protein-silver (protargol) impregnations, and that described by De Freitas (1936) was employed for the iron hematein-stained preparations.

The protein-silver technique was in a few instances modified by using Mallory's bleach prior to the impregnation. Mallory's bleach consists of treating the preparations with 0.5 per cent potassium permanganate (5 min.), washing thoroughly in distilled water, and finally treating the washed preparations with 5 per cent oxalic acid (5 min.). Subsequently the preparations are washed in distilled water and impregnated in the usual manner. The bleaching process may be used successfully on protozoa which, because of prolonged storage in alcohol between fixation and impregnation, will not take silver without it. Also, organisms in thick fecal smears impregnate much better after bleaching. The same method gave good results with organisms which under ordinary circumstances could not be impregnated (e.g., *Giardia*).

De Freitas' procedure for iron-hematein staining was slightly modified. He adjusted the hydrogen-ion concentration of the hematein solution with dibasic potassium phosphate ($K_2HPO_4$) and 1/N hydrochloric acid and used phenol red as an indicator. Since the colorimetric method of pH adjustment is lengthy, Sörensen's phosphate buffer solutions were used instead. M/30 phosphate solutions ($Na_2HPO_4$ and $KH_2PO_4$) were employed. By substitution of proper values in the simplified formula $pH = 6.8 + \log \text{acid/base}$, a solution of any desired pH could easily be prepared.

It has also been found that the 1 : 2 dilution of a saturated alcoholic (95 per cent) solution of picric acid (1 part of saturated solution of acid and 2 parts of 95 per cent alcohol) as suggested in De Freitas' method, was much too slow in destaining the flagellates. This has been observed to be true for a large number of different organisms. A full strength alcoholic solution of picric acid has been found to be most satisfactory for the purpose of destaining.

## DESCRIPTIVE ACCOUNT

### Living Material

Living specimens of *Monocercomonas verrens* when examined in darkfield appear as spheres, or more or less elongated ovoid bodies (fig. A). In general, the stouter forms predominate over the more slender ones. The largest or-

ganisms may reach about 9$\mu$ in length, whereas the smallest do not exceed 5.5$\mu$. The cytoplasm is highly granular and contains many vacuoles. The ovoid, relatively voluminous nucleus is situated near the anterior end of the flagellate. The axostyle, which in spite of many examinations could not be traced over its whole length, protrudes beyond the posterior end of the body. The protruding free portion is about 1.5$\mu$ long and appears as a slender rod, which tapers to a point; not infrequently it is extended in a filament of varying length.

The flagella, which originate at, or very close to, the anterior cell border, are morphologically and functionally differentiated into two distinct groups. One group is represented by three anterior synchronized flagella, and the

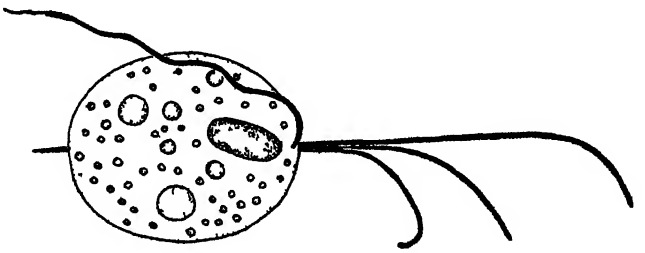

Fig. A. Camera-lucida drawing of a living specimen (very much slowed down) as observed in darkfield. The three anterior flagella originate in a columnar projection. The trailing flagellum originates posterior to the anterior group; it ends in a fine filament. The nucleus and the cytoplasmic vacuoles and granules can be clearly seen. Only the projecting portion of the axostyle is visible. × 3600.

other by a single trailing flagellum. The synchronized group originates in a short (about 1$\mu$) anterior cytoplasmic projection similar to the one described in *Pentatrichomonas hominis* (Kirby, 1943, 1945). The anterior flagella are rather stout filaments of uniform width; their ends are rounded and do not show any morphological differentiation. Their movement may be divided into three steps. In the first step the flagella are applied to the ventral surface of the body; in the second, they simultaneously and very rapidly sweep forward and upward and come to lie in line with the posterior projection of the axostyle (fig. A). In the third step the flagella, one immediately following the other, return to their original position at the surface of the body. During the last step each flagellum is characteristically curved. The concavity of the curvature faces the direction of movement. This movement of the anterior flagella in *Monocercomonas verrens* much resembles the situation found in *Pentatrichomonas hominis* (Kirby, 1943, 1945).

The trailing flagellum originates close to, but slightly posterior to, the synchronized group. In its length and thickness it is closely comparable to the anterior flagella; however, its movements are completely independent, and it ends in a very fine filament, which varies in length (fig. A). The trailing flagellum is turned posteriad and it moves much like the terminal flagellum found on the border of the undulating membrane in the genus *Trichomonas*. Since it is sometimes closely applied to the body of the cell, and since its movements are usually restricted to the dorsal surface of the organism, this

flagellum may be interpreted on a superficial examination as a border of an undulating membrane.

The dying flagellates exhibit striking changes in viscosity of the cytoplasm, which becomes very liquid. Since the viscosity of the cytoplasm usually decreases before the organelles are lost, the undulating movements of the cytoplasm caused by the beating of the trailing flagellum may be observed.

In a few organisms I was able to observe grains of rice starch in the cytoplasm. Thus it seems that *Monocercomonas* is capable of ingesting solid food particles. I have not, however, observed any structure which suggests a cytostome.

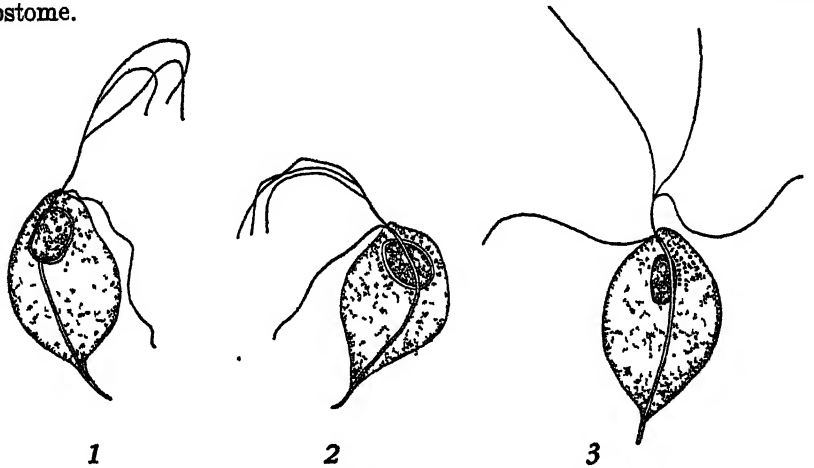


Fig. B, 1–3. Camera-lucida drawings of specimens fixed in Schaudinn's fluid and stained with iron hematein. All specimens show three anterior flagella, which are fused at the base; the trailing flagellum; the single blepharoplast; the nucleus with its membrane (1, 2); the endosome (1); the chromatic granules around the nucleus; the axostyle; and the cytoplasmic vacuoles and granules. × 3600.

## Fixed and Stained Preparations

The body of *Monocercomonas verrens* is ellipsoidal or pyriform (fig. B, 1, 2, 3; fig. C, 1, 2, 3). A hundred individuals on four slides ranged in length from 5.3 to 8.6$\mu$, in width from 3.0 to 7.9$\mu$, averaging 7.1 × 8.6$\mu$. Anteriorly the body is rounded with the exception of a small protuberance in the region of origin of the anterior flagella (fig. B, 1, 2, 3). The posterior part of the body is usually drawn out for some distance proximal to the point at which the axostyle projects from the cytostome. The free projecting portion of the axostyle ranges from 1.1 to 2.5$\mu$ (sometimes 3.0$\mu$), averaging 1.6$\mu$.

In preparations impregnated with protein silver the cytoplasm remains light and fails to show any differentiation. In iron hematein-stained preparations, on the other hand, it appears to be filled with vacuoles, which vary in size and number. The vacuoles usually do not contain any solid particles; sometimes, however, bacteria may be observed within them.

The three anterior flagella usually adhere to one another for considerable distances and not infrequently for their whole length (fig. C, 1). In the specimens where the flagella were separated for most of their length, this separa-

tion was never observed to start at their base, the flagella being invariably adherent for a short distance. In protein silver-impregnated specimens this part appears as a solid black column about 1$\mu$ long (fig. C, 2, 3, 4; fig. D, 2, 3, 5). This arrangement is comparable to that found by Kirby in *Pentatrichomonas hominis*. It seems possible that in living specimens this column is enveloped by the anterior cytoplasmic protuberance. The flagella are on the average about 1.9 times longer than the body. They stain uniformly throughout their whole length. In protein silver-impregnated specimens the terminal parts of the anterior flagella frequently show more or less distinct knoblike enlargements (fig. C, 1, 2, 3). These enlargements vary greatly in size and shape. They may appear either spherical or elongated. Sometimes, and this is especially true for the elongated ones, the terminal parts of the flagella may be bent at a slight angle (fig. C, 3). In several specimens one or more anterior flagella were observed to end in a very fine short filament (fig. C, 5). No such terminal differentiation of the anterior flagella could be seen in the iron hematein-stained preparations.

The single trailing flagellum equals the anterior flagella in length and apparently also in thickness. It impregnates uniformly with protein silver. At the end of this flagellum there is always a very fine filament which varies considerably in length (Peitschengeissel of Vlk, 1938). As with the anterior flagella, the terminal differentiation of the trailing flagellum could not be observed in specimens stained with iron hematein.

All four flagella find their origin in the blepharoplast complex, which in most specimens is hidden beneath a deeply impregnated, membranelike structure, the pelta. A suggestion of the pelta may be observed in Dobell's (1907) figures of *Trichomastix* (= *Monocercomonas*) *serpentis* (= *colubrorum*). As far as could be ascertained in the present investigation and from the literature, the iron hematoxylin- and iron hematein-stained specimens of *Monocercomonas* fail to show the structure with any clarity.

Up to the present time the pelta has been described by Kirby (1945) in protein silver-impregnated preparations of *Pentatrichomonas hominis* and by Kozloff (1945) in similarly treated specimens of *Trichomonas limacis*. In *M. verrens* the pelta is situated at the anterior periphery of the body. Its main crescentic portion (fig. C, 1, 3; fig. D, 1, 2, 3, 4, 5), the convex edge of which frequently covers the anterior border of the cytosome, sends out two extensions. One of them runs dorsally to the nucleus. It is usually a slender, long rod, which tapers to a point (fig. D, 1, 2). Sometimes, however, it appears to be of equal diameter throughout its whole length (fig. D, 3). In a few specimens the dorsal extension of the pelta curved toward the nucleus (fig. D, 1). In many preparations this structure cannot be observed, because it either fails to impregnate properly, or, still more frequently, is hidden beneath the anterior portion of the trailing flagellum. Sometimes the position of the dorsal extension is merely suggested by a row of unevenly impregnated granules. The other extension of the pelta is situated in the region ventral to the nucleus (fig. D, 1, 2, 3, 4, 6). It is considerably shorter than the dorsal extension. Sometimes it tapers to a point (fig. D, 1); more frequently, however, its terminal

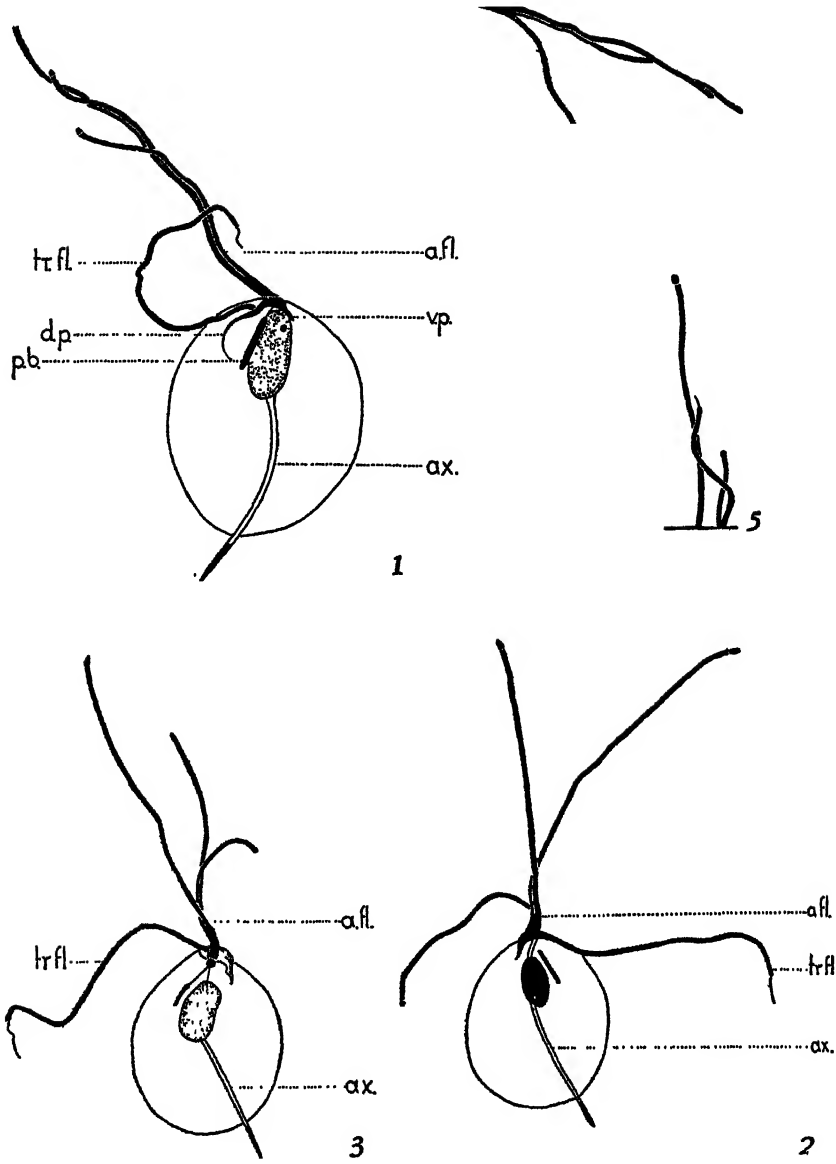


Fig. C. Camera-lucida drawings of specimens fixed in Hollande's cupric picroformol and impregnated with protein silver (protargol). × 3600. 1. An unusually large specimen. The anterior flagella are applied to one another for a considerable distance. They show the terminal knoblike differentiations. The trailing flagellum, turned forward, shows the terminal filament. (For further details see fig. D, 1.) 2. The three anterior flagella are separated for the whole length. The columnar projection from which they originate may be clearly seen. Their terminal differentiations are rod-shaped. The trailing flagellum (to the observer's right) ends in a fine filament. The axostyle may be traced above the nucleus. Its terminal portion is characteristically deeply impregnated. 3. The anterior flagella are separated for almost their whole length. They originate in a columnar projection. The trailing flagellum (to the observer's right) terminates in a fine filament. (For details see fig. D, 5.) 4. A detached mastigont. Only three anterior flagella and pelta are shown. 5. Ends of the anterior flagella. Middle flagellum terminates in a filament. Remaining flagella have the usual endings.

Abbreviations: a.fl. = anterior flagella, ax. = axostyle, bl. = blepharoplast, d.p. = dorsal extension of pelta, pe. = pelta, p.b. = parabasal body, rh. = rhizoplast, tr.fl. = trailing flagellum.

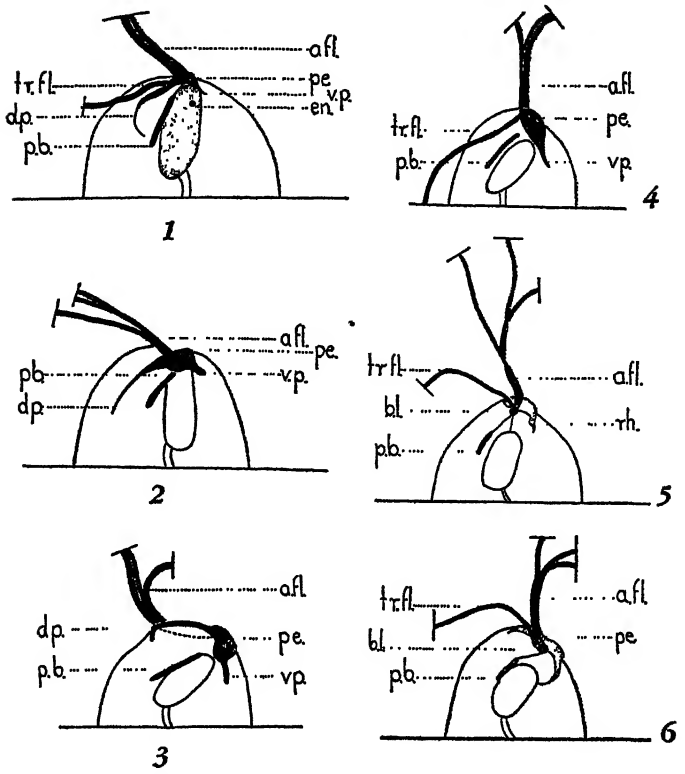


Fig. D, 1–6. Camera-lucida drawings of mastigont structures of the anterior part of the body. In all specimens the anterior flagella originate in a columnar projection, which later divides into three flagella (not seen in 1). The trailing flagellum is shown only in 1, 4, 5, 6. In 1–4 the pelta lies over the body; in 5–6 under the body. Hollande's cupric picroformol. Bodian protein silver (protargol). × 3600. 1. The dorsal extension of pelta is clearly visible, it curves toward the dorsal part of the nucleus. The ventral extension of pelta is represented by a very short projection at the upper ventral region of the nucleus. The parabasal body overlaps the anterodorsal part of the nucleus. The fine filament originating at the anterior end of the parabasal body disappears under the pelta to join the blepharoplast complex. The nucleus shows the endosome. 2. The dorsal extension of pelta runs dorsally to the nucleus. The ventral extension of pelta lies above the anteroventral side of the nucleus. The parabasal body runs dorsally and at a sharp angle to the nucleus and covers its anterodorsal part; it ends under the pelta. 3. The stout dorsal extension of pelta lies very close to the periphery of the body. The ventral extension of pelta is turned posteriad and faces the ventral side of the nucleus. The parabasal body overlaps the anterodorsal portion of the nucleus. 4. The dorsal extension of pelta is not visible. The ventral extension of pelta runs posteriad and faces the ventral surface of the nucleus. The parabasal body runs parallel to the nucleus. 5–6. The blepharoplast complex is represented by a solid granule. The anterior flagella originate in a single column at the anterior portion of the blepharoplast. The trailing flagellum originates somewhat behind the anterior group on the dorsal side of the blepharoplast. A fine filament connects the blepharoplast with the parabasal body. The rhizoplast connects the blepharoplast with the anterior end of the nucleus. The dotted area in the background represents the pelta, which is situated under the body.

portion is rounded (fig. D, 2, 3, 4). In several specimens the ventral extension appeared to curve toward the nucleus (fig. D, 6). As suggested by Kirby (1945), who reported this structure for the first time, the pelta, although it "has an integrity of its own," may possibly constitute a part of the capitulum of the axostyle.

The blepharoplast complex, which could be studied in most of the iron hematein-stained specimens and in a few silver-impregnated ones, is a single granule situated very close to the anterior border of the cell (fig. B, 1, 2, 3; fig. C, 3; fig. D, 5, 6). The three anterior flagella originate in the anterior part of the blepharoplast and the trailing flagellum originates in the posterior and dorsal part of the blepharoplast (fig. D, 5, 6). A slender fiber connects the blepharoplast complex with the parabasal body, and another filament, the rhizoplast, runs from it to the nuclear membrane (fig. D, 5, 6).

The voluminous ellipsoidal nucleus ranges in length from 1.7 to 3.6$\mu$, in width from 0.9 to 2.2$\mu$, averaging 2.5 × 1.7$\mu$. It is situated in the anterior part of the cytosome, just posterior to the pelta, and its anterior end is not infrequently covered posterior to this structure (fig. D, 1, 2). The detailed structure of the nucleus could be studied only in the iron hematein-stained specimens. The nuclear membrane, although thin, may be easily observed in many specimens (fig. B, 1, 2). The chromatin material appears to be rather uniformly distributed throughout the nucleus, and larger chromatin aggregates are found only rarely. The endosome, as observed in some specimens, is a large perfectly spherical granule surrounded by a clear zone of nucleoplasm (fig. B, 1; fig. D, 1).

In the cytoplasm near the nucleus there are a large number of chromatic granules which vary greatly in their size and arrangement. They may form one or more rows dorsal to the nucleus (fig. B, 3) or may surround it completely (fig. B, 2). Although the granules are usually more numerous in the vicinity of the nucleus, they are by no means restricted to the anterior part of the cytosome (fig. B, 1, 2, 3).

The parabasal body, which was never observed in the iron hematein-stained preparations, is shown with clarity in most specimens impregnated with protein silver. It is a stout rod of uniform thickness, situated dorsally to the nucleus. Its length varies from about one-half to about three-quarters of that of the nucleus (fig. D, 1, 2, 3, 4, 5). Sometimes the parabasal body is placed at an angle to the nucleus (fig. D, 1, 2, 3). In other instances it is placed parallel to the nucleus (fig. C, 2; fig. D, 4, 5).

The axostyle is a slender hyaline rod, which originates in the blepharoplast complex and runs to the right side of the nucleus. It then traverses the cytosome, and emerges at the posterior end of the body. No anterior enlargement of the axostyle has ever been observed. In its course through the cytoplasm the axostyle is either straight or slightly bent. The free projecting part is usually straight and tapers gradually to a point. The distal three-quarters of this projecting part impregnate much darker than the rest of the axostyle (fig. C, 1, 2, 3). No suggestion of an axostylar ring can be found at the point where the rod leaves the cytoplasm.

In spite of a careful search no trace of the cytostome could be found in either hematein-stained or protein silver-impregnated preparations.

## Monocercomonas verrens sp. n.

*Type host.*—*Tapirus malayanus* Raffles, 1821. Malayan Peninsula. Kept in San Francisco Zoölogical Gardens.

*Type slides.*—In Collection of the University of California.

*Diagnosis.*—The diagnosis of *Monocercomonas verrens* sp. n. is based on the observations of iron hematein-stained and silver-impregnated preparations as well as on examinations of living specimens.

Body pyriform or ovoid, size range: 5.3–8.6 × 3.0–7.9$\mu$, average 7.1 × 5.1$\mu$. Three anterior flagella and one trailing flagellum. All flagella equal in length and width, 1.9 times longer than the body. Anterior flagella fused at the base, originate in a columnar cytoplasmic extension, exhibit a synchronized sweeping movement. All flagella originate in a single blepharoplast complex. Blepharoplast partly or totally covered by the pelta. Pelta crescentic, with dorsal and ventral extensions, situated at the anterior end of the body. Nucleus anterior, oval, voluminous, size range 1.7–3.6 × 0.9–2.2$\mu$, average 2.5 × 1.7$\mu$, with a distinct membrane, chromatin material uniformly distributed; connects with blepharoplast by a rhizoplast. Parabasal body rod-shaped. Axostyle slender, uniform in thickness; runs from blepharoplast to the right side of the nucleus; traverses cytosome and protrudes posteriorly by about 1.6$\mu$.

## SUMMARY

In the fecal material of *Tapirus malayanus* there was found a flagellate, *Monocercomonas verrens* sp. n.

In the living specimens studied in darkfield the three anterior flagella originate in a single column and exhibit a synchronized sweeping movement. The trailing flagellum, exhibiting an undulating movement, originates somewhat posterior and dorsal to the anterior group and ends in a fine filament.

In fixed and stained preparations the body of the flagellate averages 5.3 × 8.6$\mu$. At the anterior end of the body there is a small protuberance in the region of the origin of the three anterior flagella. These anterior flagella are fused at the base. The trailing flagellum is equal in length and width to the anterior flagella, and it ends in a very fine filament. All the flagella are about 1.9 times longer than the body. They originate in a single, anteriorly situated blepharoplast complex, which in the protein silver-treated specimens is usually hidden underneath a membranelike structure, the pelta. The pelta is crescentic in shape and sends out a dorsal and a ventral extension. This structure has hitherto not been described in the genus *Monocercomonas*. The anteriorly situated nucleus averages 2.5 × 1.7$\mu$. It shows a definite thin membrane and frequently a large endosome. The chromatin is uniformly distributed throughout the nucleus. A fine rhizoplast connects the nuclear membrane with the blepharoplast complex. The parabasal body is rod-shaped and runs dorsal to the nucleus. It is connected to the blepharoplast complex by a fine filament. The axostyle is a slender rod, which runs from the blepharoplast complex along the right side of the nucleus and, after traversing the cytoplasm, emerges at the posterior end of the body. The projecting part of the axostyle averages 1.6$\mu$. The cytoplasm is highly vacuolated and contains many chromatic granules. These granules are particularly dense in the vicinity of the nucleus where they may be arranged in rows or may surround the nucleus. Sometimes bacteria may be found in the vacuoles.

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# CILIATES FROM THE SIERRA NEVADA BIGHORN, OVIS CANADENSIS SIERRAE GRINNELL

BY

MILDRED BUSH and C. A. KOFOID

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# CILIATES FROM THE SIERRA NEVADA BIGHORN OVIS CANADENSIS SIERRAE GRINNELL

BY

MILDRED BUSH AND C. A. KOFOID

## INTRODUCTION

THE CILIATES with which this paper deals were taken from the stomach of a Sierra Nevada bighorn, *Ovis canadensis sierrae* Grinnell (1913). The bighorn was taken on the east slope of Mount Baxter, near Independence, California, on October 20, 1911, by H. A. Carr, who was collecting for the Museum of Vertebrate Zoölogy of the University of California. The contents of the stomach were preserved and given to one of us (C. A. Kofoid) for study.

The bighorn from which the ciliates were taken became the type specimen of *Ovis cervina sierrae*, described by Grinnell in 1912. The specimen, consisting of skin, horns, and complete skeleton, is No. 16360 in the collection of the Museum of Vertebrate Zoölogy.

## MATERIALS AND METHODS

The ciliates, taken from the stomach immediately after the sheep was shot, were killed and preserved in formalin. For study, temporary mounts were made in glycerine, in water (one half tap and one half distilled) tinged with eosin, and in chlor-zinc-iodide solution. This method of mounting made it possible to roll the ciliates about so that all parts could be observed. In the water, osmotic action expanded the organisms so that ciliary lines were easily traced. Chlor-zinc-iodide solution was used specifically for skeletal plates. Permanent mounts were stained with Heidenhain's haematoxylin (alcoholic method) for study of ciliary lines, fibers, and nuclear structure. Some material was mounted in celloidin and stained with Best's carmine for study of skeletal plates. All drawings were made with a camera lucida.

## CILIATES OF THE FAMILY OPHRYOSCOLECIDAE

### *Entodinium nanum* sp. nov.

(Plate 24, figure 1)

*Diagnosis.*—Body small, ovoid, widest anteriorly; anterior end truncated in contracted individuals; posterior end rounded with a short ventral lobe; macronucleus short, stout club-shaped. Length 18–26 microns, 10 specimens.

*Description.*—In contracted individuals, the anterior end is truncated with the equal adoral lips barely protruding. The posterior end is rounded with only a short ventral lobe. All other surfaces are convex. The oesophagus is large in proportion to the body, turns dorsad, and terminates at the level of the posterior third of the body. The endoplasmic sack is outlined by a thin boundary. The ectoplasm forms a relatively thick layer around the endoplasmic sack and fills the short, posterior ventral lobe. The short rectum turns dorsad and opens

at the anus, which is just dorsal to the ventral lobe. The macronucleus is short, stout, club-shaped, largest anteriorly, and has a ventral depression. It appears ovoid when seen from the dorsal side. The relatively large, spherical micronucleus is situated in the ventral depression of the macronucleus or close to it. The contractile vacuole is anterior and to the left of the macronucleus. The food in the endoplasmic sack consists of small particles.

*Variation.*—This species showed little variation in the individuals observed. Some of the specimens were somewhat narrow for their length and rounded posteriorly so that the ventral lobe was hardly discernible.

*Measurements.*—Based on 10 specimens taken at random.

| Axis | Microns | Ratio to dorsoventral diameter |
|---|---|---|
| Body length | 21 (18–26) | 1.45 (1.28–1.55) |
| Transdiameter | 9 (8–11) | 0.63 (0.57–0.66) |
| Dorsoventral diameter | 14 (12–17) | 1.00 |
| Macronucleus | 6 (5–7) | 0.40 (0.31–0.51) |

*Occurrence.*—*Entodinium nanum* occurs frequently in the stomach contents of the material studied.

*Relationships.*—*Entodinium nanum* is almost the same size as *E. exiguum* Dogiel (1925*b*) and *E. nanellum* Dogiel (1923). It differs from *E. exiguum* in that its lateral outline narrows somewhat posteriorly and ends in a slight ventral lobe, whereas *E. exiguum* has almost the same width throughout its entire length and lacks the lobe. Its macronucleus is stout and club-shaped while that of *E. exiguum* is short, thick, and of equal diameter throughout. *Entodinium nanum* differs from *E. nanellum* in that its anterior end in contracted forms is truncated, while that of the other species is convex with a depression on the ventral side. The former ciliate is shorter in proportion to its dorsoventral diameter (1.45) than is *E. nanellum* (2.00), and its stout macronucleus is also shorter in proportion to the diameter (0.31–0.53) than is the wedge-shaped macronucleus of *E. nanellum* (0.71–1.11).

### *Entodinium orbicularis* sp. nov.

(Plate 24, figure 2)

*Diagnosis.*—Orbicular, all sides convex; slightly compressed laterally; posterior end smoothly rounded, no lobes or spines; macronucleus ellipsoidal, anterior to middle of body. Length 21–36 microns; dorsoventral width 17–30 microns; 10 specimens.

*Description.*—All surfaces are strongly convex. The posterior end is smoothly rounded and without lobes or spines. In contracted specimens, the oral apparatus is small and completely retracted so that the adoral lips do not protrude beyond the convex curve of the anterior end. The mouth is a small, round opening. The oesophagus is comparatively broad and extends slightly dorsad to the center of the body. The endoplasmic sack is orbicular in shape with its outlines smoothly convex except at a depression around the macronucleus. The thin ectoplasmic layer is of equal thickness around the endoplasmic sack in all parts except where it thickens in the region of the macronucleus. The rectum is short, cylindrical, and extends slightly dorsad to the anus, which is at the center of the posterior surface. The ellipsoidal macronucleus is near the dorsal

wall, just anterior to the middle of the body. The large, spherical micronucleus is ventral to the macronucleus. The food consists of small particles which could not be identified.

*Variation.*—The specimens observed did not vary appreciably in any important morphological feature or in proportions.

*Measurements.*—Based on 10 specimens taken at random.

| Axis | Microns | Ratio to dorsoventral diameter |
|---|---|---|
| Body length | 29 (22–33) | 1.20 (1.11–1.25) |
| Transverse diameter | 18 (13–24) | 0.64 (0.55–0.70) |
| Dorsoventral diameter | 25 (18–32) | 1.00 |
| Macronucleus | 6 (4–7) | 0.20 (0.18–0.24) |

*Occurrence.*—*Entodinium orbicularis* occurs rarely in the material studied.

*Relationships.*—*Entodinium orbicularis* is closely related to *E. bovis* Wertheim (1935*a*). It is slightly shorter (22–33 microns) than *E. bovis* (26.25–38.5 microns), is not so nearly round in lateral outline, and is less compressed transversely. The ectoplasm is not so highly developed in the anal region as it is in *E. bovis*. The macronucleus is short and ellipsoidal, whereas that of *E. bovis* is elongate and thickened at the anterior end. The micronucleus of *E. orbicularis* is relatively larger than that of *E. bovis*.

### *Entodinium protuberans* sp. nov.

(Plate 24, figure 3)

*Diagnosis.*—Body elongate, ovoid, widest anteriorly; all surfaces slightly convex, more so near the ends; adoral lips in contracted forms protruding and bent dorsad, the dorsal lip shorter; one short, posterior, ventral lobe; macronucleus ellipsoidal, near middle of body at middorsal line. Length 20–45 microns, 10 specimens.

*Description.*—The greatest diameter is at 0.20 of the total length from the anterior end, and from this point, all surfaces curve anteriorly or taper posteriorly. The diameter at the posterior end is about 0.50 of the greatest diameter. At the anterior end of contracted forms, the adoral lips characteristically protrude about 0.15 of the dorsoventral diameter and bend dorsad. The dorsal lip is shorter and bends at a wide acute angle. This oral structure is conspicuous, making it easy to distinguish this species from other species of *Entodinium* in the stomach contents. The dorsal surface rounds smoothly to the anus, but the ventral surface terminates posteriorly in a short, rounded lobe. In lateral view the two surfaces come together at the anal opening and form almost a right angle, a pronounced character in this species. The mouth opens into the oesophagus, which extends back to the middle of the body. The endoplasmic sack is marked by a feebly defined outline close to the body wall and occupies most of the body. The ectoplasm forms a thin layer around the endoplasmic sack and fills the posterior lobe. The rectum is short, cylindrical, and opens at the anus, which is just dorsal to the posterior lobe. The short, ellipsoidal macronucleus is on the middorsal line about the middle of the body. The spherical micronucleus is ventral to the macronucleus. The contractile vacuole is anterior to the macronucleus and opens at the middorsal line.

The endoplasmic sack is filled with small particles of plant tissue.

*Variation.*—This species varies considerably in length. The smaller individuals, apparently newly divided forms, tend to be broader in proportion to their length than the larger ones. The morphological features are constant. The animal is easily distinguished by the protruding, bent, adoral lips in contracted forms, the tapering sides, and the right angle at the posterior end.

*Measurements.*—Based on 15 specimens taken at random.

| Axis | Microns | Ratio to dorsoventral diameter |
|---|---|---|
| Body length | 27.5 (20–45) | 1.80 (1.44–2.20) |
| Transdiameter | 7.5 (6–12) | 0.54 (0.50–0.60) |
| Dorsoventral diameter | 15.0 (11–27) | 1.00 |
| Macronucleus | 7.0 (5–8) | 0.38 (0.27–0.47) |

*Occurrence.*—*Entodinium protuberans* occurs in great numbers in the material studied.

*Relationships.*—In this host *Entodinium protuberans* is near *E. simplex* Dogiel (1925*b*) in size; large individuals of the former and small individuals of the latter may have the same length. *E. simplex* lacks conspicuous adoral lips. *E. simplex* has almost the same diameter throughout its entire length; *E. protuberans* tapers posteriorly. *E. simplex* is rounded at the posterior end and the anus is slightly ventral; *E. protuberans* has a short lobe and the anus is slightly dorsal. The macronucleus of *E. simplex* is elongate and widens at the anterior end, whereas that of *E. protuberans* is ellipsoidal or spheroidal.

## *Entodinium simplex* Dogiel, 1925

*Entodinium parvum* Buisson; Dogiel, 1926

*Diagnosis.*—Body oblong-ovate; all sides smoothly convex; posterior end smoothly rounded; macronucleus slender, thickened at anterior end, lying within the anterior two thirds of the body. Length 35–44 microns, 10 specimens.

*Measurements.*—In the following table, measurements of 10 specimens of *Entodinium simplex* taken at random from the Sierra Nevada bighorn, are compared with measurements of the same species from other hosts as given by Dogiel (1927).

| Axis | From Sierra Nevada bighorn | | From other hosts | |
|---|---|---|---|---|
| | Microns | Ratio to dorsoventral diameter | Microns | Ratio to dorsoventral diameter |
| Body length | 38 (35–44) | 1.70 (1.68–1.75) | 43 (38–50) | 1.70–1.74 |
| Transdiameter | 15 (13–16) | 0.68 (0.60–0.80) | ..... | ..... |
| Dorsoventral diameter | 24 (21–27) | 1.00 | 25 (21–29) | 1.00 |
| Macronucleus | 13 (10–14) | 0.49 (0.48–0.51) | ..... | ..... |

*Occurrence.*—*Entodinium simplex* was first described by Dogiel (1925*b*) from the reindeer. He reported it in 1927 in sheep and cattle from various parts of Russia, and in 1934 in the Kamchatka wild sheep (*Ovis nivicola nivicola*), in

the yak, and in sheep from Mongolia. Hsiung (1931) recorded it in sheep in China, and Becker and Everett (1930) found it in lambs in the United States. It is found infrequently in the bighorn.

*Relationships.*—Wertheim (1935*a*) made a comparative study of *E. simplex*, *E. exiguum*, *E. nanellum*, *E. dubardi dubardi*, and *E. parvum*, which are all small species. In the Sierra Nevada bighorn, *E. simplex* is nearest *E. protuberans* in size and form. Comparison with that species is made in the account of *E. protuberans* (p. 240).

### *Entodinium truncatum* sp. nov.

(Plate 24, figure 7)

*Diagnosis.*—Body broadly ellipsoidal, compressed laterally; anterior end in contracted specimens truncated or slightly oblique; posterior end truncated, flattened laterally, no lobes or spines; macronucleus oblong-ellipsoidal or slightly reniform, situated at the middle of the dorsal side of the body. Length 36–50 microns, 10 specimens.

*Description.*—The sides are evenly convex. In contracted specimens, a depression is formed at the anterior end, in which the heavy ventral lip overlaps the dorsal lip so that the latter can hardly be distinguished. The part of the body posterior to the endoplasmic sack is flattened laterally. The posterior end is truncated, without spines or lobes but with the dorsal part rounded and the ventral part forming an obtuse angle. The cuticle is thin and flaccid so that the animals sometimes appear as shapeless masses. Mounting in water (one half distilled, one half tap) restores them more nearly to their normal form. The oesophagus extends backward and dorsad, ending near the posterior end of the macronucleus. The endoplasmic sack is outlined by a distinct boundary line and is surrounded by a thick layer of ectoplasm which is especially well developed on the dorsal side and at the posterior end. The long, narrow rectum extends straight back to the anal opening, which is at the center of the posterior end. The macronucleus is oblong-ellipsoidal or reniform in shape, and is situated at the middle of the body on the dorsal side. The micronucleus is spherical and is close to the ventral side of the macronucleus. The single, large contractile vacuole is anterior to the macronucleus and opens at the middorsal line. The food in the endoplasmic sack appears to consist of small particles of plant tissue.

*Variation.*—This species varies noticeably in proportions, some individuals being as broad as long. The morphological details do not vary greatly, and the animal can be identified among numerous other species because of its depressed anterior end and its lobeless, truncated posterior end.

*Measurements.*—Based on 10 specimens taken at random.

| Axis | Microns | Ratio to dorsoventral diameter |
|---|---|---|
| Body length | 46 (40–50) | 1.37 (1.17–1.66) |
| Transdiameter | 26 (20–35) | 0.62 (0.50–0.78) |
| Dorsoventral diameter | 34 (28–40) | 1.00 |
| Macronucleus | 14 (10–17) | 0.37 (0.31–0.48) |

*Occurrence.*—*Entodinium truncatum* is infrequent in the material studied.

*Relationships.*—*Entodinium truncatum* and *E. montanum* have similar anterior ends in contracted individuals and do not differ greatly in size, but the

latter is ovoid in lateral view and has a short posterior lobe, whereas the former has no lobe. The macronucleus of *E. truncatum* is oblong-ellipsoidal or reniform; that of *E. montanum* is club-shaped or sausage-shaped.

### *Entodinium montanum* sp. nov.

(Plate 24, figure 6)

*Diagnosis.*—Body elongate, ovoidal, compressed laterally; sides evenly convex; anterior end truncated in contracted forms; one short, ventral, posterior lobe; macronucleus club- or sausage-shaped, one third to one half the length of the body. Length 36–53 microns, 10 specimens.

*Description.*—The widest part of the body is just anterior to the middle. The sides are evenly convex. In contracted specimens, the anterior end is truncated. The ventral adoral lip is heavy and folds over the dorsal lip so that the latter is almost entirely obscured. The dorsal surface continues smoothly rounded to the anal opening without posterior prolongation. The ventral surface terminates in a short, smoothly rounded lobe which reaches to the anal opening. The oesophagus is long, narrow, and extends sharply dorsad to a point near the posterior end of the macronucleus. The endoplasmic sack is outlined by a thin boundary and occupies most of the body. The ectoplasm forms a thin layer on the ventral and lateral body walls but thickens on the dorsal side and in the posterior region. The cylindrical rectum is long, runs obliquely (45°) dorsad, and terminates in the anus, which is dorsal to the ventral posterior lobe. The macronucleus is club-shaped or sausage-shaped and thickens gradually toward the anterior end. It is near the dorsal wall in the anterior half of the body. The micronucleus is ellipsoidal and lies at the middle of the ventral side of the macronucleus. The single contractile vacuole is anterior to the macronucleus and opens to the left of the middorsal line. The food in the endoplasmic sack consists of small particles which could not be identified.

*Variation.*—This species varies somewhat in length but is fairly constant in body proportions and in morphology.

*Measurements.*—Based on 10 specimens taken at random.

| Axis | Microns | Ratio to dorsoventral diameter |
|---|---|---|
| Body length | 47 (36–53) | 1.63 (1.34–1.85) |
| Transdiameter | 20 (16–25) | 0.70 (0.64–0.78) |
| Dorsoventral diameter | 29 (22–32) | 1.00 |
| Macronucleus | 19 (12–25) | 0.67 (0.44–0.88) |

*Occurrence.*—*Entodinium montanum* is infrequent in the material studied.

*Relationships.*—A comparison with *E. truncatum* is given in the account of that species (p. 241–242).

### *Entodinium sierrae* sp. nov.

(Plate 25, figure 9)

*Diagnosis.*—Body elongated oblong-ellipsoidal, compressed laterally; lateral surfaces slightly convex; contracted specimens oblique at anterior end; two small lobes at posterior end; macronucleus short, ellipsoidal. Length 45–65 microns, 10 specimens.

*Description.*—This species is the largest of the genus *Entodinium* from this host. Both dorsal and ventral surfaces are slightly convex and the convexity of the latter increases around the posterior lobe. The lateral surfaces are evenly and very slightly convex. The anterior end is rounded in partly contracted forms and oblique in fully contracted ones, the dorsal side extending the farther forward. There are two posterior lobes of which the ventral one is broad, massive, and smoothly rounded while the dorsal one is short and bluntly pointed. The oesophagus is short and terminates back of the level of the posterior end of the macronucleus. The endoplasmic sack occupies most of the body. The ectoplasm forms a thin layer around the endoplasmic sack and thickens in the posterior lobes. The rectum lies at an angle of 45° from the axis and opens at the anus, which is at the junction of the two lobes. The macronucleus measures only about 0.15 of the total length. It is oblong-ellipsoidal in outline and is located dorsally, anterior to the middle of the body. The micronucleus is ellipsoidal or spheroidal and is ventral to the posterior end of the macronucleus. The single contractile vacuole is relatively small, is situated anterior to the macronucleus, and opens at the left of the middorsal line. The food consists of large and small particles of plant tissue, small species of *Entodinium*, and other ciliates.

*Variation.*—This species varies little except in body length. Short individuals, which may be newly divided forms, are found.

*Measurements.*—Based on 10 specimens taken at random.

| Axis | Microns | Ratio to dorsoventral diameter |
|---|---|---|
| Body length | 56 (45–65) | 1.60 (1.38–1.80) |
| Transdiameter | 27 (25–30) | 0.70 (0.60–0.75) |
| Dorsoventral diameter | 36 (30–40) | 1.00 |
| Macronucleus | 12 (8–16) | 0.31 (0.21–0.40) |

*Occurrence.*—*Entodinium sierrae* occurs frequently in the material studied.

*Relationships.*—*Entodinium sierrae* is similar in size and proportions to *E. anteronucleatum laeve* Dogiel (1927). Like *E. bursa* Stein (*E. vorax vorax* Dogiel, 1925), it is carnivorous.

### *Entodinium bicaudatum* sp. nov.

(Plate 24, figures 4, 5)

*Diagnosis.*—Orbicular to elliptical in lateral view, with two posterior spines; right surface strongly convex; left surface with wide depression; ventral surface terminating posteriorly in a spine with a massive base, and a short, rounded lobe; dorsal surface terminating in a curved spine with a broad, flangelike base; macronucleus ellipsoidal; length 22–34 microns; width 21–34 microns; ventral spine 9–15 microns; 15 specimens.

*Description.*—In lateral view, the body appears almost circular in outline and is usually slightly longer than broad, but sometimes it is broader than long. The dorsal surface is strongly convex, the ventral surface less convex. The ventral part of the body terminates posteriorly in a short, inconspicuous, rounded lobe on the right and a long spine on the left. This spine has a wide, massive base with a distinct, constricted distal part longer than the base and tapering to a point. The spine turns dorsad, then obliquely posteriorly. The

dorsal part of the body terminates posteriorly in a second spine with a broad, flangelike base which extends transversely across the right side of the posterior surface of the body to about the mid-point. The slender, free, distal part of the spine is flexible and is curved ventrad or to the right or left. In the lateral view the two spines appear to cross, but they are separated by the transverse width of the body. The right side of the body is smoothly convex. On the left side, there is a deep and wide depression which is dorsal to the base of the ventral spine and which extends anteriorly three fourths of the length of the body. The oesophagus is short, bends dorsad, and ends near the center of the body. The endoplasmic sack is marked by a weak boundary layer and occupies most of the body. The ectoplasmic layer is thin on the ventral, right, and left sides but thick on the dorsal side. The spines consist entirely of ectoplasm. The rectum is short and terminates at the anus, which is near the center of the posterior wall, between the ventral lobe and the base of the ventral spine. The macronucleus is short, ellipsoidal or spherical in form, and is situated dorsally in the anterior half of the body. The micronucleus is comparatively large, 0.4 the diameter of the macronucleus, and is on the posterior ventral side of the larger nucleus. The contractile vacuole is anterior and to the left of the macronucleus. Food consists of small particles of plant tissue.

*Variation.*—This species does not vary greatly in size but considerably in proportions. Specimens with dorsoventral diameter greater than the length often occur.

*Measurements.*—Based on 15 specimens taken at random.

| Axis | Microns | Ratio to dorsoventral diameter |
|---|---|---|
| Body length | 27.7 (22–34) | 1.05 (0.96–1.26) |
| Transdiameter | 14.0 (12–15) | 0.51 (0.48–0.55) |
| Dorsoventral diameter | 26.4 (21–34) | 1.00 |
| Macronucleus | 7.6 (5–12) | 0.25 (0.20–0.29) |
| Ventral spine | 12.0 (9–14) | 0.66 (0.42–0.90) |

*Occurrence.*—*Entodinium bicaudatum* occurs frequently in the material studied.

*Relationships.*—*Entodinium bicaudatum* is about the same size as *E. bicornutum* Dogiel (1925*b*) but is shorter in proportion to its dorsoventral diameter. The two species appear much alike from the dorsal side. Each has two conspicuous posterior spines, one ventral and one dorsal; but those of *E. bicornutum* cross while those of *E. bicaudatum* are separated by the transdiameter of the body. *E. bicornutum* has no posterior ventral lobe and no depression on the left side. The macronucleus of *E. bicaudatum* is short and ellipsoidal; that of *E. bicornutum* is trapezoidal from the dorsal side.

*Entodinium bicaudatum* and *E. caudatum* Stein (1859) are similar in the appearance of the anterior ends of contracted individuals, in having a depression of the left side and in having three posterior prolongations. *E. caudatum* is longer in proportion to its width. In *E. caudatum* the dorsal posterior spine is long, and the two preanal lobes are short, one sharp, the other rounded. In *E. bicaudatum* the dorsal and the preanal spines are about the same length and the preanal lobe is short and rounded.

## *Entodinium caudatum* Stein, 1859

(Plate 25, figure 8)

*Diagnosis.*—Body elongated, ovoidal; dorsal surface convex; ventral surface less strongly convex; much compressed laterally; posterior end with a long dorsal spine and two ventral lobes, the left short and bluntly rounded, the right acutely pointed.

*Variation.*—In specimens of *Entodinium caudatum* observed from this host, the length and width of body and the length of the posterior spine vary little. On the whole the animals are smaller and are longer in proportion to their width than those described from other hosts. The macronucleus is shorter in proportion to body length and to the dorsoventral diameter.

*Measurements.*—Based on 10 specimens taken at random.

| Axis | Microns | Ratio to dorsoventral diameter |
|---|---|---|
| Body length | 35.3 (32–38) | 1.45 (1.25–1.65) |
| Transdiameter | 13.0 (12–16) | 0.55 (0.40–0.65) |
| Dorsoventral diameter | 24.5 (22–30) | 1.00 |
| Macronucleus | 11.0 (8–14) | 0.39 (0.33–0.42) |
| Posterior dorsal spine | 23.9 (20–27) | 0.98 (0.80–1.22) |

*Occurrence.*—*Entodinium caudatum* was reported by Stein (1858) in sheep in Germany. Schuberg (1888) made the first drawing of it and Eberlein (1895) found it in cattle in Germany. It was reported by Cunha (1914) in cattle in Brazil, by Dogiel (1927) in cattle and sheep in Russia, by Hsiung (1931) in sheep in China, and by Becker and Talbott (1927) in cattle in America. The species occurs infrequently in the stomach contents of the bighorn studied.

*Relationships.*—Comparison with *E. bicaudatum* is made in the account of that species (p. 244).

## *Metadinium tauricum* (Dogiel and Fedorowa, 1925) Kofoid and MacLennan, 1932

*Diplodinium medium* Awerinzew and Mutafowa, 1914, var. *tauricum* Dogiel and Fedorowa, 1925, p. 100, fig. 3.

*Eudiplodinium medium* Awerinzew and Mutafowa, 1914, forma *tauricum* Dogiel and Fedorowa, 1927, p. 126–127, fig. 70.

*Metadinium tauricum* (Dogiel and Fedorowa, 1925) Kofoid and MacLennan, 1932, p. 115.

*Diagnosis.*—See Kofoid and MacLennan (1932, p. 115).

*Occurrence.*—Dogiel (1927) reported *Metadinium tauricum* to be frequent in sheep and infrequent in goats and cattle in various parts of the U. S. S. R. and Persia. Hsiung (1931) reported it in sheep in China. It occurs in abundance in the bighorn studied.

*Measurements.*—The food of this species consists of large bits of plant debris, particularly of long, vegetable fibers which distend and distort the body. Ten individuals, which contained no fibers, were measured and the results compared with those given by Dogiel (1927, p. 127).

| Axis | Length in microns | Dorsoventral diameter | Ratio to dorsoventral diameter |
|---|---|---|---|
| Sierra Nevada Bighorn | 256 (184–345) | 242 (170–300) | 1.25 (0.94–1.28) |
| From other hosts (Dogiel) | 235 (185–288) | 134 (70–160) | 1.75 |

*Variation.*—*Metadinium tauricum* from the Sierra Nevada bighorn varies considerably in size but little in proportions. In general, specimens studied were larger, and the dorsoventral diameter was greater in proportion to the length than those reported from other hosts.

## Genus *Polyplastron* Dogiel, 1927

*Polyplastron* Dogiel, 1927, pp. 130–134, figs. 73–74; 1928, partim, p. 332 (for pp. 332–334, fig. 4*a*, *b*, see *Elytroplastron* Kofoid and MacLennan, 1932, p. 119).

*Diagnosis.*—Ophryoscolecidae with dorsal and adoral membranelle zones at the anterior end of the body; two skeletal plates beneath the right surface, either separate or fused; three longitudinal plates beneath the left surface, with their anterior ends connected by transverse bars or a thickened band; a line of contractile vacuoles beneath the dorsal surface, with additional vacuoles beneath the other surfaces. (The diagnosis of the genus *Polyplastron* given by Kofoid and MacLennan, 1932, p. 116, is here revised in order to include a new species, *P. californiense*.)

*Type species.*—*Polyplastron multivesiculatum* (Dogiel and Fedorowa, 1925) from domestic cattle in the U. S. S. R.

### *Polyplastron californiense* sp. nov.

(Plate 25, figures 10, 11)

*Diagnosis.*—Body oval, stout; two right skeletal plates and three left plates, the middle one small; five or more contractile vacuoles; posterior end with a heavy, broadly rounded ventral lobe. Length 130–200 microns, 10 specimens.

*Description.*—The operculum projects anteriorly in contracted individuals. The sides are smoothly convex. The posterior end has a wide, massive ventral lobe. Two skeletal plates lie beneath the right surface. The first of these, the "primitiva," lies to the right of and close to the macronucleus. It has two lobes at the anterior end; the larger lobe extends into the operculum, and the other lies just posterior to the adoral zone. From the lobes the plate narrows to a slender shaft, curves backward, and tapers to a point near the posterior body wall. The second plate, the "carina," is shorter and wider than the first. It has two wide anterior lobes, the larger of which extends ventrad and the other extends toward the smaller lobe of the first plate. Back of the lobes, the plate tapers posteriorly for about three fourths of its length and then widens on the dorsal edge to form a terminal lobe shaped like half of an arrowhead.

Three skeletal plates occur on the left side. The middle plate, the "anticarina," is small, short, trapezoid-shaped and widest at the anterior end. The dorsalmost plate, the "tergum," is long and narrow, begins near the anterior end of the middle plate, extends dorsad, follows the dorsal wall backward, and tapers to a point near the posterior body wall. The third plate, the "scutum," begins near the anterior end of the middle plate, curves ventrad and then posteriorly, and tapers to a point at about half the body length. In some individuals, the distal part of this plate lies along the ventral wall. The three plates are connected at the anterior ends by a band of thickened ectoplasm which takes a darker stain than the surrounding protoplasm. The band usually lacks definite plate structure, but in some specimens a few skeletal prisms are formed.

The macronucleus is an elongate, slightly curved, club-shaped body which enlarges anteriorly and tapers gradually to a rounded tip at the posterior end. The ellipsoidal micronucleus lies in a depression in the macronucleus. The depression may be on the dorsal side or on the right or left surface of the macronucleus.

Five contractile vacuoles were found. Four of these lie along the middorsal line and one lies to the right near the anterior end of the macronucleus, on a level with the first dorsal vacuole. The oesophagus is relatively short. Its fibrils extend into the body to the anterior third of the macronucleus. The endoplasmic sack occupies most of the body. The ectoplasm forms a thin layer which thickens dorsally and in the ventral lobe. The rectum is a large, thick-walled funnel with heavy longitudinal fibrils; it terminates in an elliptical anus dorsal to the ventral lobe. The margin of the anus is scalloped by the thickened ectoplasms at the ends of the rectal fibers. The food consists of vegetable fibers and bits of debris.

*Variation.*—The length varies noticeably but the proportions are fairly constant. Some variation occurs in the form of the middle plate on the left side and in the band connecting the anterior ends of the three left plates. The middle plate may be sharply outlined with distinct and compact prisms, or it may be loosely formed with the prisms widely separated. The band connecting the plates on the left side is more dense in some individuals than in others, and it may contain a few skeleta[1] prisms.

*Measurements.*—Based on 10 specimens taken at random.

| Axis | Microns | Ratio to dorsoventral diameter |
|---|---|---|
| Length | 162 (130–200) | 1.21 (1.11–1.29) |
| Transdiameter | 97 (80–115) | 0.72 (0.66–0.82) |
| Dorsoventral diameter | 133 (110–155) | 1.00 |
| Macronucleus | 102 (85–115) | 0.76 (0.66–0.84) |

*Occurrence.*—*Polyplastron californiense* occurs abundantly in the material studied.

*Relationships.*—*Polyplastron californiense* is similar to *P. multivesiculatum* (Dogiel and Fedorowa, 1925), the type species, in general morphology but is larger and somewhat shorter in proportion to its dorsoventral diameter. Both have five plates similarly arranged, but the individual plates differ markedly. In *P. californiense* the first right plate is almost as long as the body, lobed at the anterior end, and curves to a point near the posterior wall. The second plate on the right is lobed at its anterior end and widens on the dorsal edge at its posterior end, which reaches almost as far back as the tip of the first plate. On the left side, the middle plate is about one sixth of the body length, trapezoid-shaped, and widest at the anterior end. The plate dorsal to the middle plate is almost as long as the body and is curved so that the distal half follows the dorsal wall back to a point near the posterior wall. The plate ventral to the middle one is half as long as the body, curves toward the ventral wall, and in some individuals the distal part of the plate lies along the ventral wall. The three left plates are connected at their anterior ends by a thickened ectoplasmic band.

In *Polyplastron multivesiculatum* the first right plate is two thirds as long as the body, is angular at the anterior end, extends straight back, and ends abruptly at about one fourth of the body length from the posterior wall. The second plate is sharply angular at the anterior end and lies parallel to and is shorter than the first plate. On the left side, the middle plate is small and similar in form to that in *P. californiense*. The plates dorsal and ventral to the middle one are short, slender, and connected at their anterior ends by rods.

### *Enoploplastron triloricatum* (Dogiel, 1925) Kofoid and MacLennan, 1932

*Diplodinium triloricatum* Dogiel, 1925*a*, pp. 133, 141, fig. 6; 1925*c*, pp. 292, 326, 347, pl. 18, figs. 65–76; Fantham, 1926, p. 568.
*Diplodinium triloricatum* forma *triloricatum* Dogiel, 1925*b*, p. 56, fig. 16.
*Ostracodinium triloricatum* forma *triloricatum* Dogiel, 1927, pp. 152–154, fig. 87.
*Diplodinium ecaudatum* Rees, 1930, pp. 369–370.
*Enoploplastron triloricatum* (Dogiel, 1925*a*) Kofoid and MacLennan, 1932, p. 141, fig. H, 1.
*Diagnosis.*—See Kofoid and MacLennan (1932, p. 141).

*Occurrence.*—*Enoploplastron triloricatum* was reported by Dogiel (1927) from cattle in the U. S. S. R., from reindeer (*Rangifer tarandus*) in northern U. S. S. R., and from antelope (*Rhaphiceros* sp.) in British East Africa; by Fantham (1926) from cattle in South Africa; by Rees (1930) from cattle in Louisiana; and by Hsiung (1931) from sheep in China. It is abundant in material studied.

*Measurements.*—The summarized measurements of this species, as given by Dogiel (1927) are: length, 60–112 microns; dorsoventral diameter 37–70 microns. Measurements of 10 specimens taken at random from the Sierra Nevada bighorn are: length, 105 microns (85–120 microns); dorsoventral diameter, 65 microns (50–80 microns); ratio of length to dorsoventral diameter, 1.70.

### *Ophryoscolex caudatus* Eberlein, 1895

*Ophryoscolex caudatus* Eberlein, 1895, pp. 247–250, pl. 16, fig. 4; Günther, 1899, pp. 545–572, figs. 1–16; Buisson, 1923, pp. 129–130, fig. 48; Fantham, 1926, p. 568; Becker and Talbott, 1927, p. 258, fig. 26.
*Ophryoscolex caudatus* forma *tricoronatus* Dogiel, 1927, pp. 199–202, figs. 110–111; Hsiung, 1931, p. 38.
*Ophryoscolex caudatus* Eberlein, 1895; Kofoid and MacLennan, 1933, p. 26.
*Diagnosis.*—See Kofoid and MacLennan (1933, p. 26).

*Occurrence.*—*Ophryoscolex caudatus* has been reported from sheep and cattle in Germany by Eberlein (1895) and by Günther (1900), from South Africa by Fantham (1926); from Iowa by Becker and Talbott (1927), from China by Hsiung (1931), from various parts of the U. S. S. R. and from Persia by Dogiel (1927). It occurs frequently in some parts of the stomach contents of the Sierra Nevada bighorn studied, and rarely in other parts.

*Measurements.*—Measurements of this species as recorded by Dogiel (1927) are: length, 150 microns (137–162 microns); dorsoventral diameter, 89 microns (80–98 microns); ratio, 1.65; spine, 54 microns (48–60 microns). Average measurements of 10 specimens from the Sierra Nevada bighorn are: length, 145 microns (135–155 microns); dorsoventral diameter, 80 microns (75–85 microns); ratio, 1.77; spine, 60 microns (50–80 microns).

### *Ophryoscolex quadricoronatus* Dogiel, 1927

*Ophryoscolex caudatus* Eberlein, 1895, forma *quadricoronatus* Dogiel, 1927, pp. 202–206, figs. 112–114.

*Ophryoscolex quadricoronatus* Dogiel, 1927; Kofoid and MacLennan, 1933, p. 26.

*Diagnosis.*—See Kofoid and MacLennan (1933, p. 26).

*Occurrence.*—Dogiel (1927) reported *O. quadricoronatus* from domestic sheep in Bokhara and Turkestan and from *Ovis orientalis cycloceros* in northern Persia. It occurs abundantly in the material studied.

*Measurements.*—Measurements of this species as recorded by Dogiel (1927) are: length 163 microns (128–180 microns); dorsoventral diameter, 96 microns (86–100 microns); ratio, 1.7. Average measurements of 10 specimens from the Sierra Nevada bighorn are: length, 143 microns (130–155 microns); dorsoventral diameter, 80 microns (78–85 microns); ratio, 1.80.

### *Ophryoscolex purkynjei* Stein, 1858

*Ophryoscolex purkynjei* Stein, 1858, p. 70; Eberlein, 1895, pp. 250–251, pl. 16, fig. 5; Braune, 1913, pp. 43–56; Cunha, 1914, pp. 58, 60, 63; Buisson, 1923, pp. 128–129, fig. 47; Dogiel and Fedorowa 1925, pp. 257–268, 2 figs.; Fantham, 1926, p. 568; Dogiel, 1927, pp. 206–210, figs. 115–116.

*Diplodinium vortex* Fiorentini, 1889, pp. 11–12, pl. 1, figs. 1–2.

*Diagnosis.*—See Kofoid and MacLennan, 1933, p. 25.

*Occurrence.*—*Ophryoscolex purkynjei* has been reported in cattle, sheep, and goats from the C. S. R. by Stein (1858); from Germany by Eberlein (1895) and Braune (1913); from various parts of the U. S. S. R. by Dogiel (1927); from South Africa by Fantham (1926); and from Brazil by Cunha (1914). It occurs rarely in the material studied.

*Measurements.*—Measurements as recorded by Dogiel (1927) are: length, 185 microns (155–215 microns); dorsoventral diameter, 95 microns (80–110 microns); ratio, 1.94. Average measurements of 5 specimens from the Sierra Nevada bighorn are: length, 171 microns (150–185 microns); dorsoventral diameter, 91 microns (75–100 microns); ratio, 1.88.

## OTHER CILIATES

The ciliates, *Dasytricha ruminantium* Schuberg, 1888, and *Isotricha prostoma* Stein, 1859, were found in the material studied, but neither was abundant.

## TABLES COMPARING THE FAUNAS OF OPHRYOSCOLECIDAE IN DOMESTIC SHEEP AND WILD SHEEP

The question whether a species of host animal has a specific infusorian, stomach fauna of Ophryoscolecidae has been discussed from time to time, but no definite decision has been reached. For comparison of faunas, in the following tables lists are given of the ciliates of this family that have been reported from various species of the genus *Ovis*.

Fifty-nine species of Ophryoscolecidae have been recorded from domestic sheep, which have been taken from many localities. Only one species, *Entodinium caudatum*, has been found in all species of Ovis that have been studied. Eleven species that have not been reported in domestic sheep have been found in wild sheep. The faunas in wild sheep are similar in regard to genera, but these genera and most of the species are found also in other host animals, such as goats and cattle.

The rather limited study of wild sheep does not indicate that species of *Ovis* have specific stomach faunas.

COMPARISON OF FAUNAS OF OPHRYOSCOLECIDAE IN DOMESTIC AND WILD SHEEP

| Species | *Ovis aries* | *O. nivicola nivicola* | *O. orientalis cycloceros* | *O. lervia* | *O. canadensis sierrae* |
|---|---|---|---|---|---|
| *Entodinium* | | | | | |
| 1. *simplex* Dogiel, 1925 | + | + | + | .. | + |
| 2. *longinucleatum* Dogiel, 1925 | + | .. | .. | .. | .. |
| 3. *longinucleatum* f. *costatum* Wertheim, 1934 | + | .. | .. | .. | .. |
| 4. *longinucleatum* f. *amphicostatum* Wertheim, 1934 | + | .. | .. | .. | .. |
| 5. *minimum* Schuberg, 1888 | + | .. | .. | + | .. |
| 6. *minimum* f. *parvicauda* Wertheim, 1935 | + | .. | .. | .. | .. |
| 7. *furca* da Cunha, 1914 | + | .. | .. | .. | .. |
| 8. *furca* f. *dilobum* Dogiel, 1927 | + | .. | .. | .. | .. |
| 9. *furca* f. *monolobum* Dogiel, 1927 | + | .. | .. | .. | .. |
| 10. *furca* f. *angustatum* Dogiel, 1927 | + | .. | .. | .. | .. |
| 11. *dubardi* Buisson, 1923 | + | .. | .. | .. | .. |
| 12. *dubardi* f. *crassicaudatum* (Buisson, 1923) Dogiel, 1927 | + | .. | .. | .. | .. |
| 13. *lobosa-spinosum* Dogiel, 1925 | + | .. | .. | .. | .. |
| 14. *bursa* Stein, 1858 | + | .. | .. | + | .. |
| 15. *vorax* f. *bispinosum* Dogiel, 1927 | + | .. | .. | .. | .. |
| 16. *ovinum* Dogiel, 1927 | + | + | + | .. | .. |
| 17. *caudatum* Stein, 1859 | + | + | + | + | + |
| 18. *dentatum* Stein, 1859 | + | .. | .. | + | .. |
| 19. *nanellum* Dogiel, 1921 | + | .. | .. | .. | .. |
| 20. *rostratum* Fiorentini, 1889 | + | .. | .. | + | .. |
| 21. *parvum* Buisson, 1923 | + | .. | .. | .. | .. |
| 22. *exiguum* Dogiel, 1925 | + | .. | .. | .. | .. |
| 23. *ellipsoideum* Kofoid and MacLennan, 1930 | + | .. | .. | .. | .. |
| 24. *bimastus* Dogiel, 1927 | + | .. | .. | .. | .. |
| 25. *triacum* Buisson, 1923, f. *dextrum* Dogiel, 1927 | + | .. | .. | .. | .. |
| 26. *nanum* sp. nov. | .. | .. | .. | .. | + |
| 27. *orbicularis* sp. nov. | .. | .. | .. | .. | + |
| 28. *protuberans* sp. nov. | .. | .. | .. | .. | + |
| 29. *bicaudatum* sp. nov. | .. | .. | .. | .. | + |
| 30. *truncatum* sp. nov. | .. | .. | .. | .. | + |
| 31. *montanum* sp. nov. | .. | .. | .. | .. | + |
| 32. *sierrae* sp. nov. | .. | .. | .. | .. | + |
| Total | 25 | 3 | 3 | 5 | 9 |
| *Eodinium* Kofoid and MacLennan, 1932 | | | | | |
| 33. *posterovesiculatum* (Dogiel, 1927) Kofoid and MacLennan, 1932 | + | .. | .. | .. | .. |
| 34. *bilobosum* (Dogiel, 1927) Kofoid and MacLennan, 1932 | + | .. | .. | .. | .. |
| Total | 2 | .. | .. | .. | .. |

COMPARISON OF FAUNAS OF OPHRYOSCOLECIDAE IN DOMESTIC AND WILD SHEEP—(*Continued*)

| Species | *Ovis aries* | *O. nivicola nivicola* | *O. orientalis cycloceros* | *O. lervia* | *O. canadensis sierrae* |
|---|---|---|---|---|---|
| *Diplodinium* | | | | | |
| 35. *quinquecaudatum* Dogiel, 1925 | + | .. | .. | .. | .. |
| 36. *dentatum* (Stein, 1858) Schuberg, 1888 | + | .. | .. | + | .. |
| 37. *anacanthum* Dogiel, 1927 | + | .. | .. | .. | .. |
| 38. *monocanthum* Dogiel, 1927 | + | .. | .. | .. | .. |
| 39. *diacanthum* Dogiel, 1927 | + | .. | .. | .. | .. |
| 40. *triacanthum* Dogiel, 1927 | + | .. | .. | .. | .. |
| 41. *tetracanthum* Dogiel, 1927 | + | .. | .. | .. | .. |
| 42. *pentacanthum* Dogiel, 1927 | + | .. | .. | .. | .. |
| 43. *anisacanthum* da Cunha, 1914 | + | .. | .. | .. | .. |
| 44. *bursa* Fiorentini, 1889 | .. | .. | .. | + | .. |
| Total | 9 | .. | .. | 2 | .. |
| *Eremoplastron* Kofoid and MacLennan, 1932 | | | | | |
| 45. *rostratum* (Fiorentini, 1889) Kofoid and MacLennan, 1932 | .. | .. | .. | + | .. |
| 46. *neglectum* (Dogiel, 1925) Kofoid and MacLennan, 1932 | + | .. | .. | .. | .. |
| 47. *bovis* (Dogiel, 1925) Kofoid and MacLennan, 1932 | + | .. | .. | .. | .. |
| 48. *dilobum* (Dogiel, 1927) Kofoid and MacLennnan, 1932 | + | .. | .. | .. | .. |
| Total | 3 | .. | .. | 1 | .. |
| *Eudiplodinium* | | | | | |
| 49. *maggii* (Fiorentini, 1889) Dogiel, 1927 | + | .. | .. | .. | .. |
| 50. *maggii* (Fiorentini, 1889) f. *costatum* Wertheim, 1933 | + | .. | .. | .. | .. |
| Total | 2 | .. | .. | .. | .. |
| *Diploplastron* Kofoid and MacLennan, 1932 | | | | | |
| 51. *affine* (Dogiel and Fedorowa, 1925) Kofoid and MacLennan, 1932 | + | + | + | .. | .. |
| Total | 1 | 1 | 1 | 1 | .. |
| *Metadinium* | | | | | |
| 52. *medium* Awerinzew and Mutafowa, 1914 | + | .. | .. | .. | .. |
| 53. *tauricum* (Dogiel and Fedorowa, 1925) Kofoid and MacLennan, 1932 | + | + | .. | + | + |
| Total | 2 | 1 | .. | 1 | 1 |

COMPARISON OF FAUNAS OF OPHRYOSCOLECIDAE IN DOMESTIC AND WILD SHEEP—(*Concluded*)

| Species | *Ovis aries* | *O. nivicola nivicola* | *O. orientalis cycloceros* | *O. lervia* | *O. canadensis sierrae* |
|---|---|---|---|---|---|
| *Polyplastron* | | | | | |
| 54. *multivesiculatum* (Dogiel and Fedorowa, 1925) Dogiel, 1927 | + | + | + | .. | .. |
| 55. *californiense* sp. nov. | .. | .. | .. | .. | + |
| Total | 1 | 1 | 1 | .. | 1 |
| *Ostracodinium* | | | | | |
| 56. *gracile* (Dogiel, 1925) Kofoid and MacLennan, 1932 | + | .. | .. | .. | .. |
| Total | 1 | .. | .. | .. | .. |
| *Enoploplastron* Kofoid and MacLennan, 1932 | | | | | |
| 57. *triloricatum* (Dogiel, 1925) Kofoid and MacLennan, 1932 | + | .. | + | .. | + |
| Total | 1 | .. | 1 | .. | 1 |
| *Epidinium* | | | | | |
| 58. *caudatum* (Fiorentini, 1889) Kofoid and MacLennan, 1933 | .. | .. | .. | + | .. |
| 59. *ecaudatum* (Fiorentini, 1889) Kofoid and MacLennan, 1933 | + | .. | .. | + | .. |
| 60. *quadricaudatum* (Sharp, 1914) Kofoid and MacLennan, 1933 | + | .. | .. | .. | .. |
| 61. *parvicaudatum* (Awerinzew and Mutafowa, 1914) Kofoid and MacLennan, 1933 | + | .. | .. | .. | .. |
| 62. *hamatum* (Schulze, 1924) Kofoid and MacLennan, 1933 | + | .. | .. | .. | .. |
| 63. *eberleini* (da Cunha, 1914) Kofoid and MacLennan, 1933 | + | .. | .. | .. | .. |
| Total | 5 | .. | .. | 2 | .. |
| *Ophryoscolex* | | | | | |
| 64. *buissoni* (Dogiel, 1927) Kofoid and MacLennan, 1933 | + | .. | + | .. | .. |
| 65. *bicinctus* (Dogiel, 1927) Kofoid and MacLennan, 1933 | + | .. | .. | .. | .. |
| 66. *purkynjei* Stein, 1858 | + | .. | .. | + | + |
| 67. *inermis* Stein, 1858 | + | .. | .. | + | .. |
| 68. *caudatus* Eberlein, 1895 | + | + | .. | + | + |
| 69. *bicoronatus* (Dogiel, 1927) | + | .. | .. | .. | .. |
| 70. *quadricoronatus* (Dogiel, 1927) Kofoid and MacLennan, 1933 | + | .. | + | .. | + |

COMPARISON OF THE NUMBER OF GENERA AND SPECIES OCCURRING IN *Ovis aries* AND *Ovis canadensis sierrae*

| | *O. aries* | *O. canadensis sierrae* | Common to both | Total |
|---|---|---|---|---|
| Number of genera | 12 | 5 | 5 | 12 |
| Number of species | 59 | 15 | 7 | 67 |
| Number of new species | 0 | 8 | 0 | 8 |

NUMBER OF GENERA AND SPECIES IN *Ovis canadensis sierrae* IN COMMON WITH OTHER SPECIES OF WILD SHEEP

| | Genera | Species |
|---|---|---|
| *O. nivicola nivicola* | 4 | 4 |
| *O. orientalis cycloceros* | 4 | 4 |
| *O. lervia* | 3 | 4 |

## SUMMARY

1. The stomach contents from one specimen of the Sierra Nevada bighorn, *Ovis canadensis sierrae* Grinnell, contained fifteen species of ciliates of the family Ophryoscolecidae, and two species of holotrichous ciliates.

2. Eight new species of Ophryoscolecidae are found in the Sierra Nevada bighorn, as follows: *Entodinium nanum*, *Entodinium orbicularis*, *Entodinium protuberans*, *Entodinium truncatum*, *Entodinium montanum*, *Entodinium sierrae*, *Entodinium bicaudatum*, and *Polyplastron californiense*.

3. The diagnosis of the genus *Polyplastron* is revised to include the species, *Polyplastron californiense*.

4. Tables comparing the faunas of Ophryoscolecidae found in domestic and wild sheep are given.

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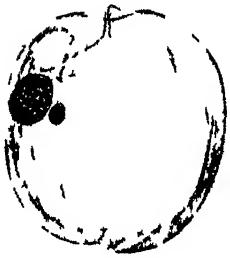
# PLATES

## PLATE 24

All figures were drawn with camera lucida from whole mounts in water tinged with eosin, × 1100

Fig 1 *Entodinium nanum* sp nov
Fig 2 *Entodinium orbicularis* sp nov
Fig 3 *Entodinium protuberans* sp nov
Fig 4 *Entodinium bicaudatum* sp nov Left lateral view
Fig 5 *Entodinium bicaudatum* sp nov Right lateral view
Fig 6 *Entodinium montanum* sp nov
Fig 7 *Entodinium truncatum* sp nov

1



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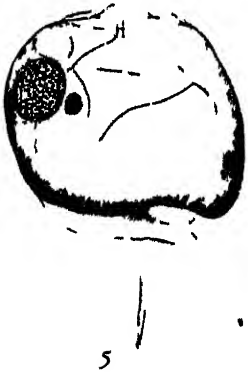

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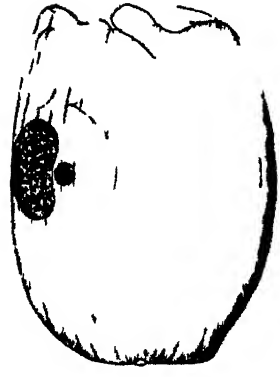

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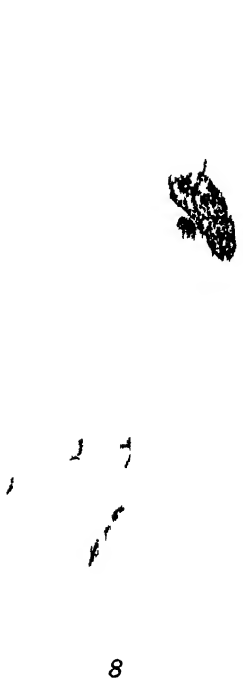
## PLATE 25

All figures were drawn with camera lucida Figures 8 and 9 were from whole mounts in water tinged with eosin × 1100 Figures 10–11 were drawn from whole mounts stained with chlor-zinc-iodide × 433

Fig 8 *Entodinium caudatum* Stein
Fig 9 *Entodinium stentae* sp nov
Fig 10 *Polyplastron californiense* sp nov Left lateral view
Fig 11 *Polyplastron californiense* sp nov Right lateral view



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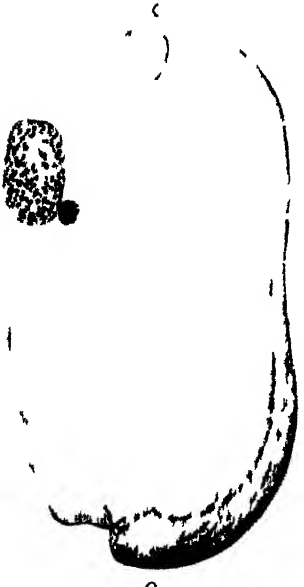

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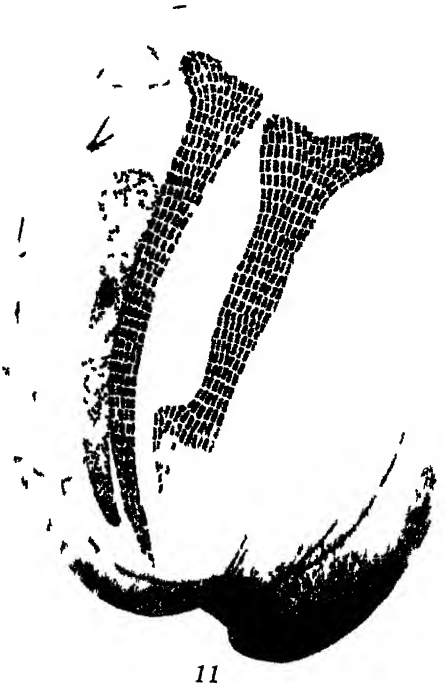

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13 MAY 1949

# CELL DIVISION IN ENTAMOEBA GINGIVALIS

BY
ELMER R. NOBLE

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# CELL DIVISION IN ENTAMOEBA GINGIVALIS

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LITTLE AGREEMENT is found in the published literature concerning the details of the process of mitosis in *Entamoeba gingivalis*. The nuclei are small, and division stages are difficult to find and to recognize in the usual smear preparation. The present investigation attempts to add more details to our knowledge of the mitotic process and nucelar cytology of this common protozoan parasite of the human mouth.

## TECHNIQUE

Amoebae were obtained in direct smears from the mouths of six students and faculty members. Smears were treated with Schaudinn's, Heidenhain's Susa, or Gilson-Carnoy fixatives, and were stained in Heidenhain's iron hematoxylin or in Harris' alum hematoxylin. Following the suggestion of Stabler (1932), the writer tried using different percentages of glacial acetic acid in the Schaudinn's fluid. The "typical" appearance of the nucleus was obtained after adding 5 per cent acetic acid to the fixative. The Feulgen reaction, with 0.02 per cent indulin as the counterstain, was employed in an effort to determine the origin of chromosomes.

## THE RESTING PHASE

(Pl. 26, figs. 1, 2, 4, 5; pl. 28, fig. 12)

The interphase nucleus varies considerably in its appearance. A typical resting condition is here described as found in smears stained in Heidenhain's iron hematoxylin after Heidenhain's Susa fixative. Peripheral chromatin is thin and occurs irregularly as blobs or beads against the inner wall of the delicate nuclear membrane. Chromatin is sometimes absent from one side, and the boundary of the nucleus is consequently difficult to distinguish there from the adjacent cytoplasm. A large blob of peripheral chromatin is frequently observed (pl. 26, fig. 4), apparently formed by fusion of much of the normal peripheral material, for when it is present the rest of the peripheral chromatin is generally thinner than usual. On these occasions the endosomal granules are often more widely separated than in the majority of truly interphase nuclei; hence the formation of such a blob would seem to be associated with the beginning of the prophase.

The periendosomal granules are minute, occupying all the zone between the peripheral chromatin and the endosome; but they are generally more concentrated about midway between these two areas. These granules may be evenly spaced or may be clustered to form irregular, pale gray patches.

The endosome consists of several deeply staining granules in the center of the nucleus and imbedded within a lighter matrix. Occasionally it is subcentral in position. Three or four granules of slightly different sizes are usual. A clear halo around the endosome is sometimes evident. Clearly defined radial threads from the endosome to the periphery were seldom observed. When present (in heavily stained material), the radii usually project from the endo-

some for a short distance, then disappear in the nucleoplasm before reaching the peripheral chromatin. There is no evidence of a centriole.

When other techniques are employed, the nuclear structures assume more varied aspects. With Schaudinn's fluid containing 5 per cent acetic acid, the resting nucleus appears much the same as that described above except that the large lateral blob of peripheral chromatin is not common and the endosome granules are more compact. With Schaudinn's fluid containing less than 5 per cent acetic acid, the endosome usually appears as a single central granule. With 20 per cent acetic acid, the nucleus is apparently rendered less capable of taking the stain (iron hematoxylin), for both peripheral chromatin and endosome are frequently observed to be pale even in heavily stained material. The peripheral chromatin sometimes does not stain at all, making the whole nucleus difficult to locate in the cytoplasm. The endosome in these slides is generally composed of three or four distinctly separated granules. The cytoplasm is more vacuolated than after the fixative with 5 per cent acetic acid. It is of interest to note that, after using 20 per cent acetic acid in Schaudinn's fluid, Stabler (1932) found the endosomes of trophozoites and cysts of *Endolimax nana* lose their stainability; and Wenrich (1937) observed the same phenomena in cysts of *Iodamoeba bütschlii*.

When smears are fixed in Gilson-Carnoy fluid, the cytoplasm is vacuolated, more shrinkage occurs than with Schaudinn's or Heidenhain's Susa fixatives, and the endosome normally appears as a single solid granule. With the Feulgen nucleal reaction, the peripheral chromatin is negative and always takes the counterstain. The periendosomal chromatin is seldom distinct enough to be discerned, but occasionally it appears to be pale pink, indicating a positive reaction. The endosome is either definitely positive (red) or not clearly visible. Harris' alum hematoxylin (after Schaudinn's fluid plus 5 per cent glacial acetic acid) gives a picture somewhat similar to that obtained by the use of iron hematoxylin after Schaudinn's fluid plus 20 per cent acetic acid, but the peripheral chromatin always appears distinct as a narrow circle in the former stain. No large blobs were observed, and the endosome appears as scattered granules, frequently three in number but often more numerous. The more compact the endosome the less distinct the periendosomal zone.

The cytoplasm has been adequately described by several writers (Kofoid and Swezy, 1924*a*; Child, 1926) and repetition here is unnecessary. No correlation appears between the number of ingested leucocyte nuclei and the stage of mitosis, except that the larger amoebae are more frequently found dividing and the larger amoebae generally contain numerous leucocyte nuclei in various stages of digestion. Small amoebae are commonly observed without leucocyte nuclei, and enucleate forms are occasionally encountered. The amoeba averages 15$\mu$ in diameter (range, 8 to 50), and its nucleus averages 3.5$\mu$ in diameter (range, 1.8 to 6.0).

## THE PROPHASE

(Pl. 26, figs. 3, 6, 7; pl. 27, fig. 8; pl. 28, figs. 13–31; pl. 29, figs. 32–36)

Most of the nuclei observed in the process of mitosis were in the prophase, indicating that this phase of division is of relatively long duration. Earliest

indications of the prophase are enlargement of the nucleus and clumping of periendosomal granules, followed by a dispersal of the endosome. Periendosomal granules gradually become clustered about the endosome granules.

Peripheral chromatin varies considerably in its behavior during the prophase. In many nuclei it changes very little from the resting appearance. In others it is more irregularly distributed, often becoming more heavily concentrated on one side (pl. 26, figs. 3, 7; pl. 28, fig. 26; pl. 29, fig. 33). As the prophase continues, the scattered endosomal and the concentrated periendosomal granules together form a large irregular mass in the center of the nucleus (pl. 28, figs. 22–31; pl. 29, figs. 32–33). Because both the endosome and chromosomes have the same staining reactions with hematoxylin and with the Feulgen technique, a distinction between early chromosomes and endosomal products is difficult at this stage. In some nuclei, however, the endosomal granules become less distinct and more circumscribed than usual and can thus be distinguished from the developing chromosomes (pl. 28, fig. 24). This difference in appearance may be related to a difference in function.

Plate 27, fig. 8, and pl. 29, figs. 34–36, are considered to represent late prophase. In this period the central chromatin granules become less numerous, pale, and scattered; and they appear to be connected by crisscross strands. Similar-appearing nuclei have been called "anaphase" by Stabler (1940) in his paper on mitosis in *Entamoeba gingivalis*. Similar figures of *E. histolytica* were labeled "prophase" by Kofoid and Swezy (1925) and by Kofoid (1927). Stabler's last prophase (his figure 4) could easily be confused with his first anaphase (fig. 5). The chief difference between the two lies in the peripheral chromatin. The latter, however, has been shown to be exceedingly variable during the prophase.

## THE METAPHASE

(Pl. 29, figs. 37–39)

No stage was observed which could unquestionably have been called a metaphase. Most nuclei which suggest the metaphase are slightly oval in shape, are pale, and have scarcely discernible fibrils, which often lie parallel to the long axis of the nucleus. These nuclei contain no heavy granules and their peripheral chromatin is thin. They would have been identified as poorly stained resting nuclei were it not that the other cellular structures in each amoeba were normally stained. One such nucleus (pl. 29, fig. 39) was observed to possess five pale strands, each with a slight, pale, central enlargement. But the parts were too indistinct for indisputable identification.

Hence it appears that between the prophase and anaphase of mitosis the nuclear elements become either achromatic or so finely divided that the stained granules are rendered indistinct. No evidence of polar caps, an intradesmose, or astral rays was observed. It is significant that Stabler also was unable to find an obvious metaphase in this amoeba. Probably a typical metaphase does not exist.

## THE ANAPHASE

(Pl. 29, figs. 40–41)

Early anaphase stages have not been identified. It is possible that the metaphase overlaps the anaphase. In late anaphase the chromosomes and fibers become aligned in the center, parallel to the long axis of the nucleus. No typical spindle is evident, but each group of chromosomes lies against the elongated nuclear membrane, and they remain connected by fibers which cross at the center of the nucleus.

In the anaphase stage peripheral chromatin becomes thin, especially at the poles of the elongating nucleus, but it persists as a slightly thickened band around the middle. In optical section this band appears as two dark stripes opposite each other in equatorial position. Stabler (1940) has described additional stainable material in the center during late anaphase and early telophase.

The chromosomes are difficult to distinguish because of their small size and their manner of formation and separation. Their number appears to be five. Five distinct chromatin bodies have often been observed in the center of the nucleus, in slides stained in iron hematoxylin and in the Feulgen preparations. But these bodies were probably the endosome in early prophase stages before condensation of periendosomal granules (pl. 28, figs. 17, 23, 27). The clearest evidence for five chromosomes is found in late anaphase. Child (1926) described six chromosomes, and Stabler (1940) described five in *Entamoeba gingivalis*.

## THE TELOPHASE

(Pl. 27, figs. 9–11)

Early telophase stages have not been observed. Later stages show two completely separated nuclei in a cell which has not commenced cytoplasmic division. The cytoplasm then divides to produce the two daughter cells, each with one of the new nuclei. Stabler (1940) has described an early telophase during which the two daughter nuclei are connected by a filament.

## DISCUSSION

Generative chromatin (idiochromatin) in *Entamoeba gingivalis* appears to be confined to periendosomal granules. A similar condition has been described for other parasitic amoebae (see Kofoid and Swezy, 1924*b*, on *Karyamoebina falcata;* Kirby, 1927, on *Endamoeba disparata;* Meglitsch, 1940, on *Endamoeba blattae;* Wenrich, 1940, on *Entamoeba muris*). But Kofoid and Swezy (1925) and Uribe (1926) stated that in *Entamoeba histolytica* the chromosomes are derived from the periendosomal chromatin and the peripheral chromatin. Child (1926) also described a dual origin of chromosomes in *E. gingivalis*. The Feulgen definitely negative reaction of the peripheral chromatin, however, would seem to suggest that this chromatin plays no part in chromosome formation.

If we restrict the definition of kinetochromatin to that chromatin which constitutes the division center (centriole) or its product, the centrodesmose,

*Entamoeba gingivalis* has no visible kinetochromatin. Neither centrioles nor astral rays are produced. This condition raises the question of the function of the endosome. The Feulgen technique colors both periendosomal granules and the endosome red. Hence the endosome and its surrounding granules may be assumed to contain thymonucleic acid. Wenrich (1940) found that in *E. muris* the endosome could be distinguished from periendosomal granules by the use of haemalum. This stain colored the granules, but the endosome was negative. In *E. gingivalis,* however, no marked distinction of this nature was observed by the writer (using Harris' alum hematoxylin).

The mingling of endosomal granules with periendosomal granules during the prophase might suggest that the endosome takes part in the formation of chromosomes. Such a function of the endosome has been described for other amoebae (see Chalkley, 1936, on *Amoeba proteus*). In the foregoing discussion of the prophase of *Entamoeba gingivalis,* however, evidence of the functional separation of endosomal and periendosomal granules was presented. Wenrich (1940) found that in *E. muris* the endosome gives rise to an intradesmose and to strands to which the chromosomes are attached. This function of the endosome was also described by Kofoid and Swezy (1925) and by Uribe (1926) for *E. histolytica.* Wenrich (1940) suggested that the endosome might be related to the kinetoplast of the Trypanosomatidae and the Cryptobiidae. In species of amoebae, however, in which the endosome does not form a division center this relationship is not evident. If, in addition to thymonucleic acid, the endosome also contains some plastin material, its function may be to aid in the formation of chromosome sheath substance, as has been suggested for the endosome (karyosome) of the Myxosporidia (Noble, 1944). The endosome may play a role in the exchange of energy and materials between parts of the nucleus and between the nucleus and cytoplasm during mitotic division.

The localization of peripheral chromatin (trophochromatin) in the central periphery of the elongating anaphase suggests the interzonal material of *Endamoeba blattae* (Morris, 1936; Meglitsch, 1940) and that of *Entamoeba muris* (Wenrich, 1940). In the latter species this material takes the form of a second set of "chromosomes." Wenrich suggested that these second chomosomes may be fundamentally equivalent to nucleolus-like bodies of *Zelleriella intermedia* described by Chen (1936). The central band of cloudy material in the anaphase of *E. histolytica* as described by Uribe (1926) may represent, at least in part, some of the peripheral chromatin, although it may be derived entirely from the endosome.

Peripheral chromatin thus tends to accumulate in the center of the anaphase figures of these amoeba. In *Entamoeba gingivalis* this tendency is relatively slight, but in other amoebae it is evidently strong enough to result in much detachment from the nuclear membrane, after which this detached peripheral chromatin may assume various well-defined forms.

Thus we find that in several respects the nucleus of *Entamoeba gingivalis* exhibits less complexity than the nuclei of many other species of amoebae. First, the metaphase plate is apparently absent. This condition may represent an accelerated mitosis during which time the prophase chromosomes divide

and separate without becoming aligned in an equatorial plate, or it may represent either a degenerate process or a primitive one. Secondly, if the endosome functions only in the exchange of energy and materials between cellular structures, this function might be considered a fundamental one, and any tendency in other amoebae to form a division center would be a secondary function of the endosome. Lastly, the periendosomal chromatin exhibits only a slight tendency to accumulate in the center of the anaphase nucleus. This behavior suggests a more primitive condition than that found in such species as *Entamoeba muris* and *Endamoeba blattae*.

## SUMMARY

The interphase nucleus of *Entamoeba gingivalis* consists of a thin layer of irregularly beaded peripheral chromatin, minute periendosomal granules, and a central endosome composed of several granules in a close cluster.

Mitosis begins with an enlargement of the nucleus, followed by a condensation of periendosomal granules and a breaking up and dispersal of the endosome. The central granular mass gradually loses its stainability; hence the only stage which might represent a metaphase appears as a pale nucleus without clearly defined chromatic bodies. Therefore the details of changes between prophase and middle anaphase have not been observed. The late anaphase consists of an elongated nucleus with two groups of chromosomes connected by fibers which cross in the center of the nucleus. Peripheral chromatin, variable in quantity during the prophase, is reduced in the anaphase, and generally is restricted to a band in the middle periphery of the dividing nucleus. Complete division of the nucleus occurs before the cytoplasm divides, and the nuclear membrane remains intact during mitosis. There is no evidence of centrioles, astral rays, polar caps, or an intradesmose. Chromosomes are apparently derived from the periendosomal granules, but the function of the endosome has not been determined. The number of chromosomes seems to be five.

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# PLATES

Figures 1–11 represent entire cells of *Entamoeba gingivalis*. The dark bodies in the cytoplasm are leucocyte nuclei which have been ingested, and which are in various stages of digestion. All were fixed in Heidenhain's Susa fluid; figures 1–9 were stained in Heidenhain's iron hematoxylin, and 10–11 were stained in Harris' alum hematoxylin. The drawings were made with the aid of a camera lucida. Magnification, × 3500.

Figures 12–41 represent nuclei only of *Entamoeba gingivalis*. Figures 16, 37, 38 were fixed in Schaudinn's fluid containing 5 per cent glacial acetic acid, and were stained in Heidenhain's iron hematoxylin; 41 was fixed in Heidenhain's Susa fluid and stained in Harris' alum hematoxylin. All the rest were fixed in Heidenhain's Susa and stained in iron hematoxylin. Magnification, × 7700.

## PLATE 26

Fig. 1. Interphase showing single endosomal granule and granular periendosomal granules.

Fig. 2. Interphase with endosome composed of 5 granules. Periendosomal material not visible.

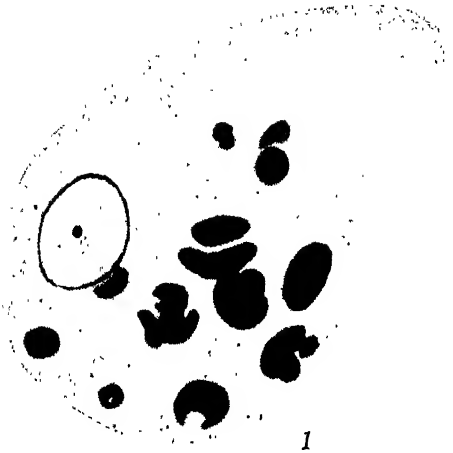
Fig. 3. Early prophase with condensing periendosomal granules.

Fig. 4. Interphase with large endosome composed of several granules, and with a large blob of peripheral chromatin.

Fig. 5. Interphase with two blobs of peripheral chromatin.

Fig. 6. Prophase showing condensation of periendosomal granules and their intermingling with endosomal granules.

Fig. 7. Prophase showing strands connecting central chromatin granules to periphery of nucleus.



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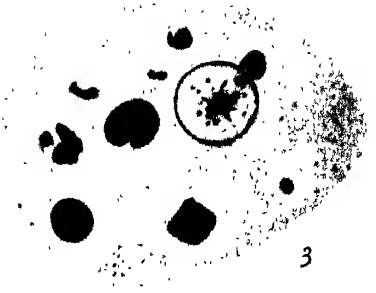

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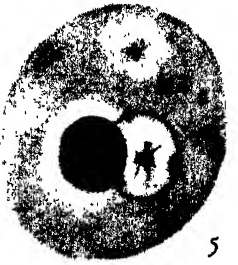

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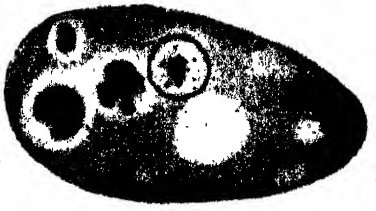

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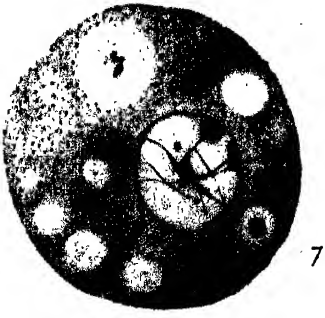

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## PLATE 27

Fig 8 Late prophase The chromatin and strands have begun to pale

Fig 9 Telophase with the two daughter nuclei resembling stages between prophase and anaphase

Fig 10 Telophase before cytoplasmic construction

Fig 11 Telophase showing cytoplasmic construction

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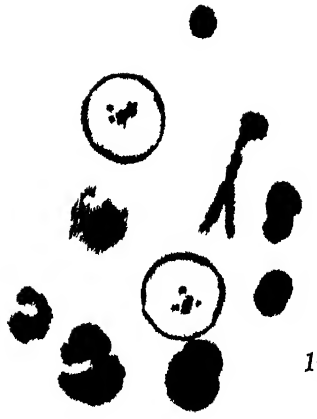

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## PLATE 28

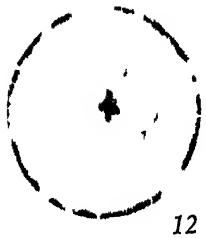
Fig. 12. Typical interphase nucleus.

Figs. 13–17. Early prophase showing condensation of periendosomal granules and separation of endosomal granules.

Figs. 18–23. Later prophase showing intermingling of periendosomal granules with those of the endosome.

Fig. 24. Unusual formation of separate idiochromatin on the left, and endosomal material on the right.

Figs. 25–31. Later prophase showing variations of the central mass. Fig. 26 shows a large lateral blob of peripheral chromatin. The central chromatin mass gradually becomes pale as its granules are dispersed.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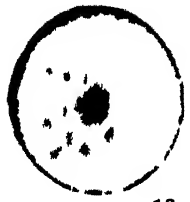
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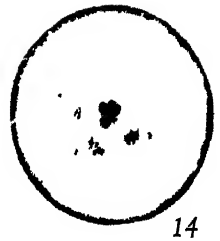
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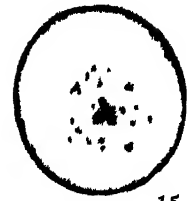
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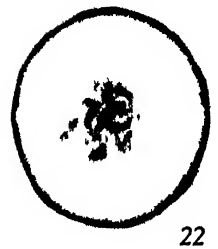
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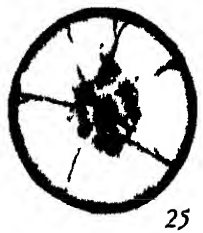
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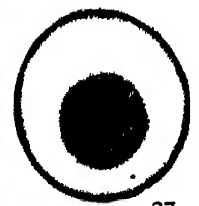
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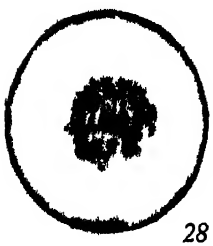
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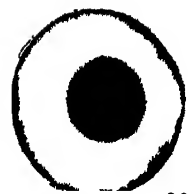
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## PLATE 29

Figs 32–33 Late prophase A continuation of changes as shown at the end of plate 28

Figs 34–36 Late prophase Central chromatin granules become less numerous, scattered, pale, and connected by crisscross fibers

Figs. 37–39 Metaphase (?) showing pale strands without conspicuous granules

Figs. 40–41 Late anaphase Fibers connecting the two groups of chromosomes cross in the middle of the nucleus Peripheral chromatin persists at the center

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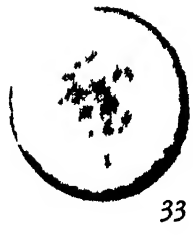
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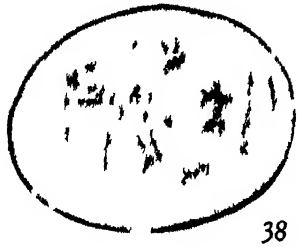
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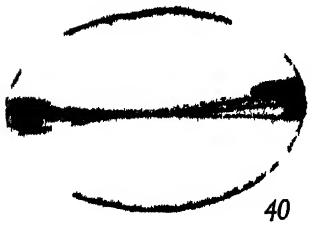
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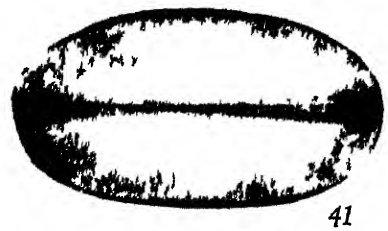
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# CELL DIVISION IN ENTAMOEBA GINGIVALIS

BY

ELMER R. NOBLE

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# CELL DIVISION IN ENTAMOEBA GINGIVALIS

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LITTLE AGREEMENT is found in the published literature concerning the details of the process of mitosis in *Entamoeba gingivalis*. The nuclei are small, and division stages are difficult to find and to recognize in the usual smear preparation. The present investigation attempts to add more details to our knowledge of the mitotic process and nucelar cytology of this common protozoan parasite of the human mouth.

## TECHNIQUE

Amoebae were obtained in direct smears from the mouths of six students and faculty members. Smears were treated with Schaudinn's, Heidenhain's Susa, or Gilson-Carnoy fixatives, and were stained in Heidenhain's iron hematoxylin or in Harris' alum hematoxylin. Following the suggestion of Stabler (1932), the writer tried using different percentages of glacial acetic acid in the Schaudinn's fluid. The "typical" appearance of the nucleus was obtained after adding 5 per cent acetic acid to the fixative. The Feulgen reaction, with 0.02 per cent indulin as the counterstain, was employed in an effort to determine the origin of chromosomes.

## THE RESTING PHASE

(Pl. 26, figs. 1, 2, 4, 5; pl. 28, fig. 12)

The interphase nucleus varies considerably in its appearance. A typical resting condition is here described as found in smears stained in Heidenhain's iron hematoxylin after Heidenhain's Susa fixative. Peripheral chromatin is thin and occurs irregularly as blobs or beads against the inner wall of the delicate nuclear membrane. Chromatin is sometimes absent from one side, and the boundary of the nucleus is consequently difficult to distinguish there from the adjacent cytoplasm. A large blob of peripheral chromatin is frequently observed (pl. 26, fig. 4), apparently formed by fusion of much of the normal peripheral material, for when it is present the rest of the peripheral chromatin is generally thinner than usual. On these occasions the endosomal granules are often more widely separated than in the majority of truly interphase nuclei; hence the formation of such a blob would seem to be associated with the beginning of the prophase.

The periendosomal granules are minute, occupying all the zone between the peripheral chromatin and the endosome; but they are generally more concentrated about midway between these two areas. These granules may be evenly spaced or may be clustered to form irregular, pale gray patches.

The endosome consists of several deeply staining granules in the center of the nucleus and imbedded within a lighter matrix. Occasionally it is subcentral in position. Three or four granules of slightly different sizes are usual. A clear halo around the endosome is sometimes evident. Clearly defined radial threads from the endosome to the periphery were seldom observed. When present (in heavily stained material), the radii usually project from the endo-

some for a short distance, then disappear in the nucleoplasm before reaching the peripheral chromatin. There is no evidence of a centriole.

When other techniques are employed, the nuclear structures assume more varied aspects. With Schaudinn's fluid containing 5 per cent acetic acid, the resting nucleus appears much the same as that described above except that the large lateral blob of peripheral chromatin is not common and the endosome granules are more compact. With Schaudinn's fluid containing less than 5 per cent acetic acid, the endosome usually appears as a single central granule. With 20 per cent acetic acid, the nucleus is apparently rendered less capable of taking the stain (iron hematoxylin), for both peripheral chromatin and endosome are frequently observed to be pale even in heavily stained material. The peripheral chromatin sometimes does not stain at all, making the whole nucleus difficult to locate in the cytoplasm. The endosome in these slides is generally composed of three or four distinctly separated granules. The cytoplasm is more vacuolated than after the fixative with 5 per cent acetic acid. It is of interest to note that, after using 20 per cent acetic acid in Schaudinn's fluid, Stabler (1932) found the endosomes of trophozoites and cysts of *Endolimax nana* lose their stainability; and Wenrich (1937) observed the same phenomena in cysts of *Iodamoeba bütschlii*.

When smears are fixed in Gilson-Carnoy fluid, the cytoplasm is vacuolated, more shrinkage occurs than with Schaudinn's or Heidenhain's Susa fixatives, and the endosome normally appears as a single solid granule. With the Feulgen nucleal reaction, the peripheral chromatin is negative and always takes the counterstain. The periendosomal chromatin is seldom distinct enough to be discerned, but occasionally it appears to be pale pink, indicating a positive reaction. The endosome is either definitely positive (red) or not clearly visible. Harris' alum hematoxylin (after Schaudinn's fluid plus 5 per cent glacial acetic acid) gives a picture somewhat similar to that obtained by the use of iron hematoxylin after Schaudinn's fluid plus 20 per cent acetic acid, but the peripheral chromatin always appears distinct as a narrow circle in the former stain. No large blobs were observed, and the endosome appears as scattered granules, frequently three in number but often more numerous. The more compact the endosome the less distinct the periendosomal zone.

The cytoplasm has been adequately described by several writers (Kofoid and Swezy, 1924*a*; Child, 1926) and repetition here is unnecessary. No correlation appears between the number of ingested leucocyte nuclei and the stage of mitosis, except that the larger amoebae are more frequently found dividing and the larger amoebae generally contain numerous leucocyte nuclei in various stages of digestion. Small amoebae are commonly observed without leucocyte nuclei, and enucleate forms are occasionally encountered. The amoeba averages 15$\mu$ in diameter (range, 8 to 50), and its nucleus averages 3.5$\mu$ in diameter (range, 1.8 to 6.0).

## THE PROPHASE

(Pl. 26, figs. 3, 6, 7; pl. 27, fig. 8; pl. 28, figs. 13–31; pl. 29, figs. 32–36)

Most of the nuclei observed in the process of mitosis were in the prophase, indicating that this phase of division is of relatively long duration. Earliest

indications of the prophase are enlargement of the nucleus and clumping of periendosomal granules, followed by a dispersal of the endosome. Periendosomal granules gradually become clustered about the endosome granules.

Peripheral chromatin varies considerably in its behavior during the prophase. In many nuclei it changes very little from the resting appearance. In others it is more irregularly distributed, often becoming more heavily concentrated on one side (pl. 26, figs. 3, 7; pl. 28, fig. 26; pl. 29, fig. 33). As the prophase continues, the scattered endosomal and the concentrated periendosomal granules together form a large irregular mass in the center of the nucleus (pl. 28, figs. 22–31; pl. 29, figs. 32–33). Because both the endosome and chromosomes have the same staining reactions with hematoxylin and with the Feulgen technique, a distinction between early chromosomes and endosomal products is difficult at this stage. In some nuclei, however, the endosomal granules become less distinct and more circumscribed than usual and can thus be distinguished from the developing chromosomes (pl. 28, fig. 24). This difference in appearance may be related to a difference in function.

Plate 27, fig. 8, and pl. 29, figs. 34–36, are considered to represent late prophase. In this period the central chromatin granules become less numerous, pale, and scattered; and they appear to be connected by crisscross strands. Similar-appearing nuclei have been called "anaphase" by Stabler (1940) in his paper on mitosis in *Entamoeba gingivalis*. Similar figures of *E. histolytica* were labeled "prophase" by Kofoid and Swezy (1925) and by Kofoid (1927). Stabler's last prophase (his figure 4) could easily be confused with his first anaphase (fig. 5). The chief difference between the two lies in the peripheral chromatin. The latter, however, has been shown to be exceedingly variable during the prophase.

## THE METAPHASE

(Pl. 29, figs. 37–39)

No stage was observed which could unquestionably have been called a metaphase. Most nuclei which suggest the metaphase are slightly oval in shape, are pale, and have scarcely discernible fibrils, which often lie parallel to the long axis of the nucleus. These nuclei contain no heavy granules and their peripheral chromatin is thin. They would have been identified as poorly stained resting nuclei were it not that the other cellular structures in each amoeba were normally stained. One such nucleus (pl. 29, fig. 39) was observed to possess five pale strands, each with a slight, pale, central enlargement. But the parts were too indistinct for indisputable identification.

Hence it appears that between the prophase and anaphase of mitosis the nuclear elements become either achromatic or so finely divided that the stained granules are rendered indistinct. No evidence of polar caps, an intradesmose, or astral rays was observed. It is significant that Stabler also was unable to find an obvious metaphase in this amoeba. Probably a typical metaphase does not exist.

## THE ANAPHASE

(Pl. 29, figs. 40–41)

Early anaphase stages have not been identified. It is possible that the metaphase overlaps the anaphase. In late anaphase the chromosomes and fibers become aligned in the center, parallel to the long axis of the nucleus. No typical spindle is evident, but each group of chromosomes lies against the elongated nuclear membrane, and they remain connected by fibers which cross at the center of the nucleus.

In the anaphase stage peripheral chromatin becomes thin, especially at the poles of the elongating nucleus, but it persists as a slightly thickened band around the middle. In optical section this band appears as two dark stripes opposite each other in equatorial position. Stabler (1940) has described additional stainable material in the center during late anaphase and early telophase.

The chromosomes are difficult to distinguish because of their small size and their manner of formation and separation. Their number appears to be five. Five distinct chromatin bodies have often been observed in the center of the nucleus, in slides stained in iron hematoxylin and in the Feulgen preparations. But these bodies were probably the endosome in early prophase stages before condensation of periendosomal granules (pl. 28, figs. 17, 23, 27). The clearest evidence for five chromosomes is found in late anaphase. Child (1926) described six chromosomes, and Stabler (1940) described five in *Entamoeba gingivalis*.

## THE TELOPHASE

(Pl. 27, figs. 9–11)

Early telophase stages have not been observed. Later stages show two completely separated nuclei in a cell which has not commenced cytoplasmic division. The cytoplasm then divides to produce the two daughter cells, each with one of the new nuclei. Stabler (1940) has described an early telophase during which the two daughter nuclei are connected by a filament.

## DISCUSSION

Generative chromatin (idiochromatin) in *Entamoeba gingivalis* appears to be confined to periendosomal granules. A similar condition has been described for other parasitic amoebae (see Kofoid and Swezy, 1924*b*, on *Karyamoebina falcata;* Kirby, 1927, on *Endamoeba disparata;* Meglitsch, 1940, on *Endamoeba blattae;* Wenrich, 1940, on *Entamoeba muris*). But Kofoid and Swezy (1925) and Uribe (1926) stated that in *Entamoeba histolytica* the chromosomes are derived from the periendosomal chromatin and the peripheral chromatin. Child (1926) also described a dual origin of chromosomes in *E. gingivalis*. The Feulgen definitely negative reaction of the peripheral chromatin, however, would seem to suggest that this chromatin plays no part in chromosome formation.

If we restrict the definition of kinetochromatin to that chromatin which constitutes the division center (centriole) or its product, the centrodesmose,

*Entamoeba gingivalis* has no visible kinetochromatin. Neither centrioles nor astral rays are produced. This condition raises the question of the function of the endosome. The Feulgen technique colors both periendosomal granules and the endosome red. Hence the endosome and its surrounding granules may be assumed to contain thymonucleic acid. Wenrich (1940) found that in *E. muris* the endosome could be distinguished from periendosomal granules by the use of haemalum. This stain colored the granules, but the endosome was negative. In *E. gingivalis,* however, no marked distinction of this nature was observed by the writer (using Harris' alum hematoxylin).

The mingling of endosomal granules with periendosomal granules during the prophase might suggest that the endosome takes part in the formation of chromosomes. Such a function of the endosome has been described for other amoebae (see Chalkley, 1936, on *Amoeba proteus*). In the foregoing discussion of the prophase of *Entamoeba gingivalis,* however, evidence of the functional separation of endosomal and periendosomal granules was presented. Wenrich (1940) found that in *E. muris* the endosome gives rise to an intradesmose and to strands to which the chromosomes are attached. This function of the endosome was also described by Kofoid and Swezy (1925) and by Uribe (1926) for *E. histolytica.* Wenrich (1940) suggested that the endosome might be related to the kinetoplast of the Trypanosomatidae and the Cryptobiidae. In species of amoebae, however, in which the endosome does not form a division center this relationship is not evident. If, in addition to thymonucleic acid, the endosome also contains some plastin material, its function may be to aid in the formation of chromosome sheath substance, as has been suggested for the endosome (karyosome) of the Myxosporidia (Noble, 1944). The endosome may play a role in the exchange of energy and materials between parts of the nucleus and between the nucleus and cytoplasm during mitotic division.

The localization of peripheral chromatin (trophochromatin) in the central periphery of the elongating anaphase suggests the interzonal material of *Endamoeba blattae* (Morris, 1936; Meglitsch, 1940) and that of *Entamoeba muris* (Wenrich, 1940). In the latter species this material takes the form of a second set of "chromosomes." Wenrich suggested that these second chomosomes may be fundamentally equivalent to nucleolus-like bodies of *Zelleriella intermedia* described by Chen (1936). The central band of cloudy material in the anaphase of *E. histolytica* as described by Uribe (1926) may represent, at least in part, some of the peripheral chromatin, although it may be derived entirely from the endosome.

Peripheral chromatin thus tends to accumulate in the center of the anaphase figures of these amoeba. In *Entamoeba gingivalis* this tendency is relatively slight, but in other amoebae it is evidently strong enough to result in much detachment from the nuclear membrane, after which this detached peripheral chromatin may assume various well-defined forms.

Thus we find that in several respects the nucleus of *Entamoeba gingivalis* exhibits less complexity than the nuclei of many other species of amoebae. First, the metaphase plate is apparently absent. This condition may represent an accelerated mitosis during which time the prophase chromosomes divide

and separate without becoming aligned in an equatorial plate, or it may represent either a degenerate process or a primitive one. Secondly, if the endosome functions only in the exchange of energy and materials between cellular structures, this function might be considered a fundamental one, and any tendency in other amoebae to form a division center would be a secondary function of the endosome. Lastly, the periendosomal chromatin exhibits only a slight tendency to accumulate in the center of the anaphase nucleus. This behavior suggests a more primitive condition than that found in such species as *Entamoeba muris* and *Endamoeba blattae*.

## SUMMARY

The interphase nucleus of *Entamoeba gingivalis* consists of a thin layer of irregularly beaded peripheral chromatin, minute periendosomal granules, and a central endosome composed of several granules in a close cluster.

Mitosis begins with an enlargement of the nucleus, followed by a condensation of periendosomal granules and a breaking up and dispersal of the endosome. The central granular mass gradually loses its stainability; hence the only stage which might represent a metaphase appears as a pale nucleus without clearly defined chromatic bodies. Therefore the details of changes between prophase and middle anaphase have not been observed. The late anaphase consists of an elongated nucleus with two groups of chromosomes connected by fibers which cross in the center of the nucleus. Peripheral chromatin, variable in quantity during the prophase, is reduced in the anaphase, and generally is restricted to a band in the middle periphery of the dividing nucleus. Complete division of the nucleus occurs before the cytoplasm divides, and the nuclear membrane remains intact during mitosis. There is no evidence of centrioles, astral rays, polar caps, or an intradesmose. Chromosomes are apparently derived from the periendosomal granules, but the function of the endosome has not been determined. The number of chromosomes seems to be five.

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# PLATES

Figures 1–11 represent entire cells of *Entamoeba gingivalis*. The dark bodies in the cytoplasm are leucocyte nuclei which have been ingested, and which are in various stages of digestion. All were fixed in Heidenhain's Susa fluid; figures 1–9 were stained in Heidenhain's iron hematoxylin, and 10–11 were stained in Harris' alum hematoxylin. The drawings were made with the aid of a camera lucida. Magnification, × 3500.

Figures 12–41 represent nuclei only of *Entamoeba gingivalis*. Figures 16, 37, 38 were fixed in Schaudinn's fluid containing 5 per cent glacial acetic acid, and were stained in Heidenhain's iron hematoxylin; 41 was fixed in Heidenhain's Susa fluid and stained in Harris' alum hematoxylin. All the rest were fixed in Heidenhain's Susa and stained in iron hematoxylin. Magnification, × 7700.

## PLATE 26

Fig. 1. Interphase showing single endosomal granule and granular periendosomal granules.

Fig. 2. Interphase with endosome composed of 5 granules. Periendosomal material not visible.

Fig. 3. Early prophase with condensing periendosomal granules.

Fig. 4. Interphase with large endosome composed of several granules, and with a large blob of peripheral chromatin.

Fig. 5. Interphase with two blobs of peripheral chromatin.

Fig. 6. Prophase showing condensation of periendosomal granules and their intermingling with endosomal granules.

Fig. 7. Prophase showing strands connecting central chromatin granules to periphery of nucleus.

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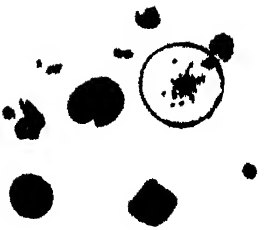

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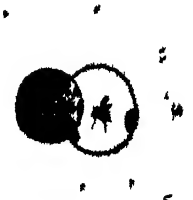

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## PLATE 27

Fig 8 Late prophase The chromatin and strands have begun to pale

Fig 9 Telophase with the two daughter nuclei resembling stages between prophase and anaphase

Fig 10 Telophase before cytoplasmic constriction

Fig 11 Telophase showing cytoplasmic constriction

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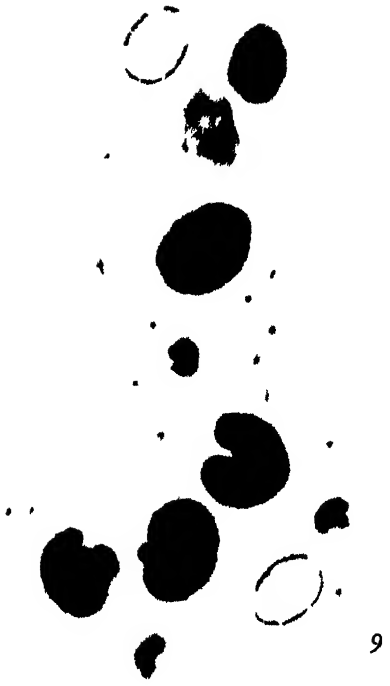

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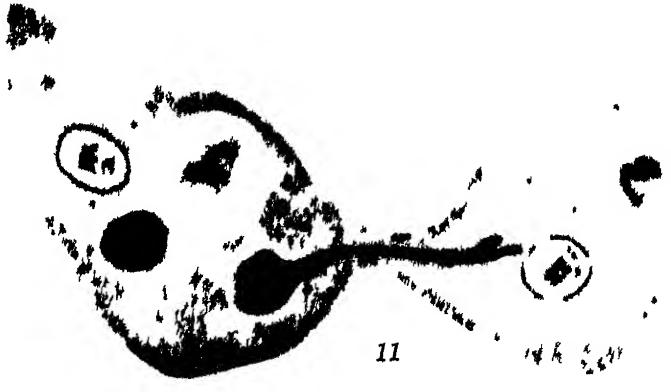

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## PLATE 28

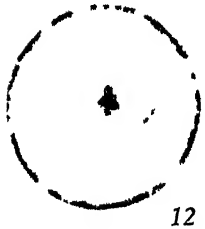
Fig. 12. Typical interphase nucleus.

Figs. 13–17. Early prophase showing condensation of periendosomal granules and separation of endosomal granules.

Figs. 18–23. Later prophase showing intermingling of periendosomal granules with those of the endosome.

Fig. 24. Unusual formation of separate idiochromatin on the left, and endosomal material on the right.

Figs. 25–31. Later prophase showing variations of the central mass. Fig. 26 shows a large lateral blob of peripheral chromatin. The central chromatin mass gradually becomes pale as its granules are dispersed.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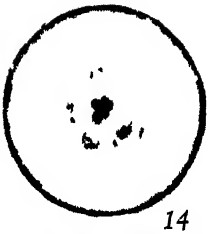
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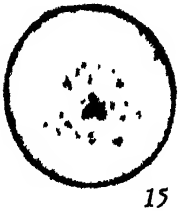
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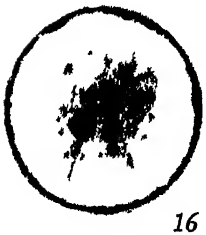
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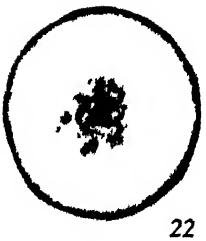
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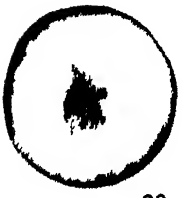
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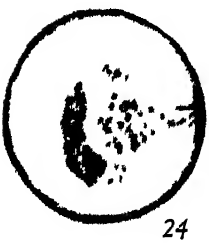
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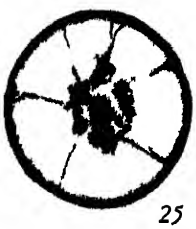
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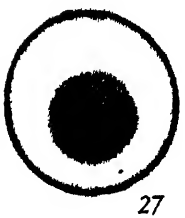
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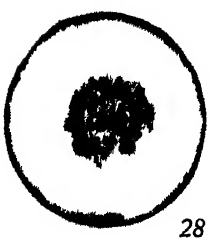
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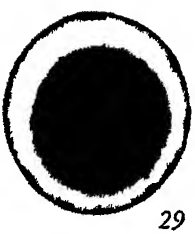
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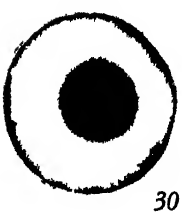
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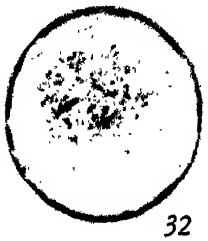
## PLATE 29

Figs. 32–33. Late prophase. A continuation of changes as shown at the end of plate 28.

Figs. 34–36. Late prophase. Central chromatin granules become less numerous, scattered, pale, and connected by crisscross fibers.

Figs. 37–39. Metaphase (?) showing pale strands without conspicuous granules.

Figs. 40–41. Late anaphase. Fibers connecting the two groups of chromosomes cross in the middle of the nucleus. Peripheral chromatin persists at the center.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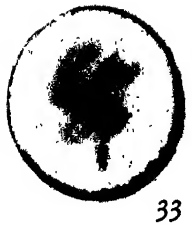
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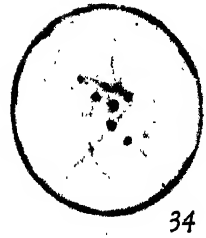
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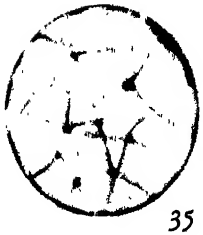
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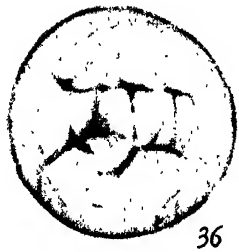
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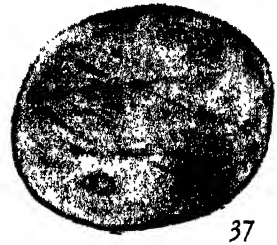
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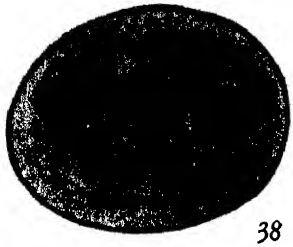
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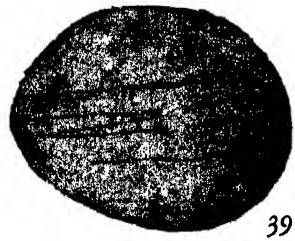
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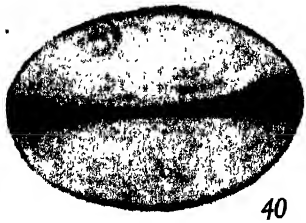
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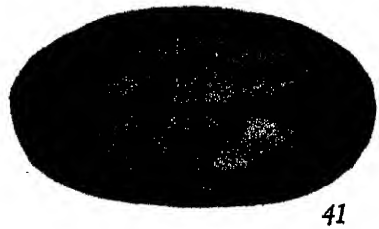
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# A MORPHOLOGICAL AND BIOCHEMICAL STUDY OF LAMPBRUSH CHROMOSOMES OF VERTEBRATES

BY

EDWARD O. DODSON

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# A MORPHOLOGICAL AND BIOCHEMICAL STUDY OF LAMPBRUSH CHROMOSOMES OF VERTEBRATES

BY

EDWARD O. DODSON

## INTRODUCTION

IN THE DEVELOPMENT of telolecithal primary oöcytes of vertebrates, the chromosomes develop many side loops, because of which they have been called lampbrush chromosomes. In the present study an attempt has been made to clarify the mode of origin of these loops, the chemical nature of the lampbrush chromosomes, their relationship to other types of chromosomes, and their probable function.

The earliest observation of lampbrush chromosomes was made by Flemming (1882), who used *Siredon* material. He noted that the chromosomes of growing eggs appeared "star-like" in cross section. Rabl figured these chromosomes in his paper of 1885, but he did not comment upon them.

In 1887 Otto Schultze published a study of lampbrush chromosomes in the amphibians *Triton cristatus, Rana fusca,* and *Siredon.* He noticed that the chromosomes lost their stainability, and hence their visibility, progressively during lampbrush formation, and that the nucleoli simultaneously became enlarged, then vacuolated, and finally broke up into fragments, some of which were filiform. His interpretation was that lampbrush formation led to destruction of the chromosomes and that more chromosomes were formed *de novo* from nucleolar material.

Rückert's paper (1892) still remains, perhaps, the best descriptive treatment of this subject. Using the elasmobranchs *Scyllium, Torpedo,* and especially *Pristiurus,* he divided the history of the chromosomes of the primary oöcyte into three phases, of which the first two are concerned with the lampbrush figure. During the first period, synapsis occurs, and the deposition of yolk begins, the growth of the cytoplasm being paralleled by growth of the nucleus and chromosomes. The chromosomes first appear as chains of microsomes, which then swell laterally and lose their stainability. He suggested that this loss of stainability might be caused either by dilution of stainable material or by chemical change, but he did not decide between these possibilities. Soon loops appear to project out into the nuclear sap from the microsomes (= chromomeres), each like a fine chromatid with its own chromomeres. He could not determine whether these loops, which in cross section formed a rosette around the main axis of the chromosome, were formed by outgrowth from the microsomes or whether they were formed from the nuclear sap, but he regarded the former hypothesis as more likely. Neither could he determine whether each loop was separate, or whether successive loops were connected to one another by unstained portions paralleling the main axis of the chromo-

some. In describing the chromosomes at this stage, Rückert introduced the term "lampbrush" which has since then been used to designate them. He thought that this structure was probably latent in the ordinary microsomes. After this figure reaches its maximum development, the chromosomes begin a reconcentration to the form in which they occur on the first maturation spindle. This process, which constitutes Rückert's second stage, he regarded as a simple reversal of the preceding stage. He estimated that the chromosomal volume increases several thousandfold in the first period and decreases by a comparable amount in the second. It is evident that the chromosomes must take up a large amount of nuclear matrix during the first period and release a comparable amount of material in the second. This release is made visible by the appearance of many small nucleoli near the chromosomes. Since the lampbrushes disappear before the maturation division, Rückert reasoned that they could not be concerned with heredity. Hence he regarded the lampbrush substance as being related to "somatic" (= trophic) functions. Most cells carry on their principal activity during the resting stage, but the immense growth of these eggs occurs during the prophase of the first maturation division. Rückert therefore regarded lampbrush formation as a means of providing a highly dispersed chromosomal form during division, a substitute for the true resting condition.

Other authors who have substantiated the work of Rückert include Born (1892), Lubosch (1902), Janssens (1904), Maréchal (1906), Loyez (1906), King (1908), and Stieve (1921). Maréchal's figures are perhaps the best that have been published. Only Carnoy and Lebrun (1897, 1898, 1899, and 1900) have supported the viewpoint of Schultze.

Ris (1945) published a descriptive paper on the lampbrush chromosomes while the present report was in preparation. His paper is primarily a study of the meiotic chromosomes of the grasshopper. He presents evidence that the chromomeres of leptotene chromosomes are simply the twists of a minor coil on a fundamentally uniform chromonema. The fuzzy appearance of the diplotene chromosomes he attributes to the development of major coils, the four chromatids separating from one another but overlapping centrally to give the appearance of a centrally located chromosome with chromomeres, and a surrounding fuzzy area. He believed that this same structure applies to the lampbrush chromosomes and he published one figure of a chromosome of a frog (species unnamed) to support his contention. The photomicrograph, however, is not clear in the critical central region, so that it is not decisive in support of his viewpoint.

The first experimental approach to the lampbrush problem was reported by Jörgensen (1913) from Goldschmidt's laboratory. He found that, when basophilic, the chromosomes of *Proteus* and of many other animals are not digestible by trypsin or pepsin, but that the oxyphilic chromosomes (as in the lampbrush stage) are readily destroyed by these enzymes. He interpreted this as meaning that the protein basis of the chromosomes is protected from the enzymes by the nucleic acid in the basophilic stages. Their digestibility during the lampbrush stage he regarded as evidence that no nucleic acid is

Koltzoff (1938) studied the lampbrush chromosomes using the Feulgen method for identification of thymonucleic acid. Animals used included *Triton*, the chicken, and the pigeon. He found that the chromosomes become completely negative before lampbrush formation and remain so until the lampbrush figure disappears before the first meiotic division. He attached great theoretical significance to this negativity, accepting it as proof that nucleic acid cannot enter into the composition of the genes. He regarded nucleic acid as a protective sheath which covers the chromosomes at times, but which must be removed if maximal chromosomal metabolism is to take place. The "genonema" or gene string he regarded as a gigantic chain molecule in which side chains function as individual genes. He thought that this genonema never enters into the reactions of the cell, lest it thus alter the hereditary characteristics, but that it serves as a contact catalyst for synthesis of materials in the adjacent karyoplasm.

Previously, Brachet (1929) had published a study of the lampbrush chromosomes of *Rana fusca*, *Triton cristatus*, and *Salamandra maculosa* using the Feulgen method. His results were similar to Koltzoff's. In a second paper (1940*a*), however, he reversed his position. He found that the main axis remains Feulgen positive at all times, although the barbs are always negative. Tissue used in his earlier studies was fixed in acetic sublimate, whereas that for the later work was fixed in Bouin's fluid, and it is to the difference in fixatives that he attributes the divergent results.

Duryee (1941) published the results of microdissection and chemical experiments on the lampbrush chromosomes of *Rana temporaria* (= *fusca*) and *Triturus pyrrhogaster*. Duryee regarded each lampbrush chromosome as "a single plastic cylinder, with imbedded granules and attached loops." In his microdissection experiments, each cylinder behaved as a single unit, thus failing to support the tetrad concept. Within each plastic cylinder, however, he found rows of paired chromioles (= chromomeres ?). He thought that the lampbrush filaments arise in the following manner: a single chromiole divides to form a pair, but the two remain connected. Now the connecting bar grows and is displaced laterally to form a lampbrush loop. In microdissection experiments Duryee stretched these chromosomes as much as 810 per cent, but the loops did not tend to flatten out: they were simply pulled farther apart. He regarded this as proof that the successive loops are not connected to one another. That lampbrush filaments actually are loops was shown by Duryee by opening out individual loops with a pair of microdissection needles. In his chemical experiments he found that the side loops are dissolved by any agent producing a pH of 8.4 or higher (see also Kodani's results). The loops he found to be digestible by both trypsin and pepsin, whereas the cylinder was digestible by trypsin only.

Clark, Barnes, and Baylor (1942) have published a study of lampbrush chromosomes by means of the electron microscope. They stated that their photographs verify earlier descriptions of the lampbrush chromosomes, but threads were never seen as loops, although some were branched and rebranched. None of their photographs was published, and the organism from

which the chromosomes were obtained was not identified, even as to higher group.

Painter (1940) has reviewed instances of intranuclear division and pointed out that in the nurse cells of *Drosophila* the chromosomal aggregates thus formed resemble lampbrush chromosomes. More by analogy to these nurse cells than by observation or experiment, he concluded that the lampbrush chromosomes of vertebrates are aggregates of thousands of chromosomes produced by intranuclear divisions. He suggested that the great majority of these are sloughed off into the cytoplasm to serve as a substrate for synthesis of cleavage chromosomes. In 1942, however, Painter and Taylor published observations on the eggs of *Bufo* stained by the Feulgen reaction and by Unna's pyronine-methyl green method for ribonucleic acid. They found the main axis to be Feulgen positive, but the side branches were negative at all times both to the Feulgen reagent and to Unna's mixture. They concluded that, since the side branches contain neither of the nucleic acids, the side branches are simply lateral expansions of matrix, and have nothing to do with the intranuclear divisions of many types of cells.

Kodani's work on the salivary gland chromosomes of *Drosophila* also has a bearing on the lampbrush problem (Kodani, 1941, 1942, 1946; Calvin and Kodani, 1941; Calvin, Kodani, and Goldschmidt, 1940; Goldschmidt and Kodani, 1942). On treatment with alkali the chromosomes shorten between the discs. Part of the nucleic acid of the discs becomes separated and is precipitated on the shunken chromonemata, making them visible. There are never more than four chromonemata visible, so that the salivary gland chromosome is basically a tetrad. The disc now appears as a group of chromatic hairs radiating around the centrally located chromonemata. A polar view always shows 8 to 12 of these hairs. It appears that the normal disc is made up of such a starlike group of chromatic hairs, together with interstitial nucleic acid which is removed by the alkali treatment and deposited on the chromonemata between the bands. Thus the alkali-treated salivary gland chromosomes simulate naturally occurring lampbrush chromosomes to a remarkable degree.

## MATERIALS AND METHODS

Ovaries of the salamander *Amphiuma means* Cuvier have been the principal material for this study; while those of the shark *Squalus suckleyi* Gill were used for comparison. Fixatives used include Bouin's, which gave the best results, Zenker's, Flemming's, Gilson's, and Karpechenko's. Sections were cut at 10$\mu$.

Some of the tissue was stained without special pretreatment, while other tissue was treated with the enzymes nuclease, pepsin, or trypsin before staining. The (thymo-) nuclease was at first prepared by the method of Mazia and Jaeger (1939). In the hope of producing a more potent solution, this was later modified by precipitating the enzyme from the concentrated solution with 10 volumes of acetone. The dried precipitate was then dissolved in a veronal buffer at pH 8.6, following the suggestion of Levene and Dillon (1933). Magnesium acetate was added in a concentration of $2.5 \times 10^{-2}$M, in order to pre-

cipitate the phosphates released from the nucleotides, as suggested by Klein (1932). Possibly because of the low solubility of the enzyme thus formed, this solution was not a marked improvement over the original one. Pepsin was used at a concentration of 1:200 in 0.2 per cent HCl, and trypsin at a concentration of 1:1000 in 0.2 per cent NaOH. Slides to be treated with proteolytic enzymes were coated with celloidin to prevent loss of sections. In all digestion experiments, the slides were incubated in the enzyme bath at 38 to 40 degrees centigrade.

Stains used included Harris' and Heidenhain's hematoxylins with orange G as a counterstain, safranin and light green, the Feulgen reagent for thymonucleic acid, and Unna's pyronine-methyl green mixture for ribonucleic acid. Thousands of eggs were stained by these procedures, using many batches of each stain. Attempts were made to stain the chromosomes by means of the ninhydrin reagent, Millon's reagent, and the biuret reagent. All these protein tests failed, however, and the failure was attributed to the extremely fine dimensions of the lampbrush chomosomes.

## OBSERVATIONS ON *AMPHIUMA MEANS*

### Normal Chromosomes

Undigested slides stained in the hematoxylins or safranin give very much the classic picture as reported by Rückert and most subsequent writers. Chromosomes of the earliest oöcytes (leptotene and zygotene) show the usual structure, with "ultimate" chromomeres upon a very fine thread. In pachytene the chromosomes become somewhat thicker, more regular bands, and this structure is carried over into early diplotene.

But soon the diplotene chromosomes assume a bristled appearance, the formerly rounded chromomeres now showing short, pointed outgrowths. These become apparent in small numbers in cells with a maximum diameter of 70 to 80$\mu$, nuclei of which have a diameter of 45 to 50$\mu$. Few if any nucleoli are visible in cells of this size. As the cell grows, these outgrowths become more and more numerous, until finally, the chromosomes have one or two such bristles on every chromomere. Such a condition is illustrated in figure 1, *a*, which was drawn from a cell with a total diameter of 140$\mu$, and a nuclear diameter of 80$\mu$. Here these bristles sometimes occur two to the chromomere, but there are many parts in which there is only one bristle per chromomere. In some of these the bristles all appear on the same side of the chromosome, whereas in others they alternate from one side to the other. A large number of small nucleoli is visible in such nuclei. Most of them are situated against the nuclear membrane, but a few may be seen in the interior.

As the bristles grow, they rapidly lose their stainability, so that they have a rather vague appearance, and their exact outlines are made out only with considerable difficulty. In a nucleus such as that shown in figure 1, *b* (cell diameter 150$\mu$, nuclear diameter 80$\mu$) several stages in the development of these pseudopod-like outgrowths may be seen. Some still appear as sharply defined bristles, similar to those illustrated in figure 1, *a*. Others have broadened and increased in length. Their fainter staining has been indicated by

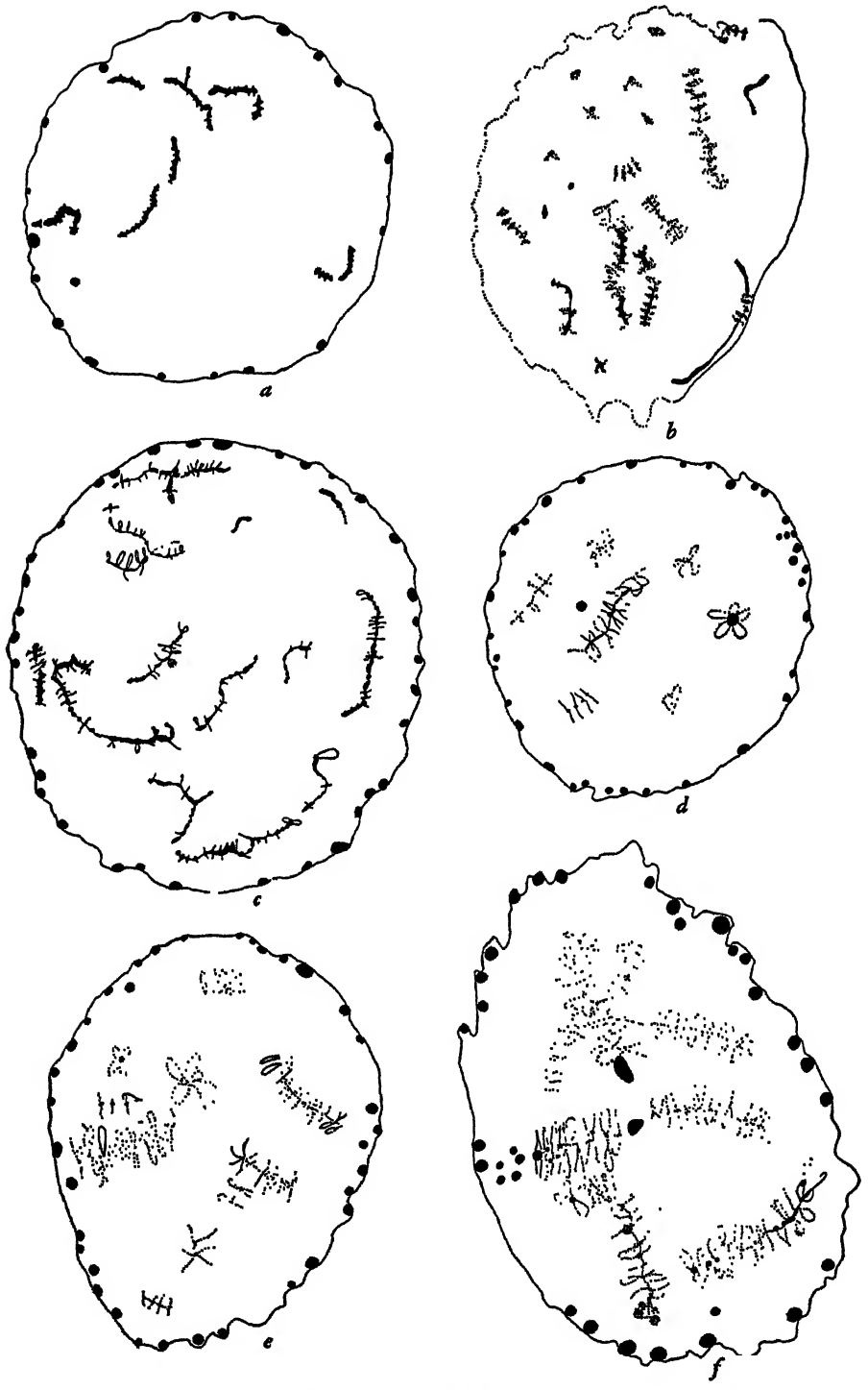


Figure 1. (For an explanation of this figure, see bottom of facing page.)

stippling, while those parts showing the typical intense hematoxylin stain are shown in solid black. These pseudopod-like processes may become broad at the base and extend out a considerable distance. They usually appear symmetrically on opposite sides of a chromosome, though occasionally unpaired outgrowths appear. In cross sections, some of the processes are single, while others are paired and symmetrical about the axis of the chromosome. A very few are unsymmetrical. A single cross section at the bottom of the figure shows four outgrowths. Finally, some of these outgrowths appear to have broken through in the center, leaving the loops which are characteristic of later stages.

Soon these loops acquire a less diffuse appearance, although they still stain very faintly. Indeed, they continue to lose in stainability for a considerable time. In the nucleus illustrated in figure 1, *c* (cell 150$\mu$, nucleus 85$\mu$), the majority of the lateral outgrowths appear as fine threads, although a few are still in the diffuse, pseudopod-like stage. Some appear to be in the bristle stage, but this may be a result of more advanced processes being cut short in sectioning. Many of the threads appear as loops, while others appear as simple threads. It seems probable, especially as two such threads frequently diverge from a common point, that all are actually loops, the simple threads being artifacts of sectioning. While these loops or other outgrowths often appear symmetrically on opposite sides of a chromosome, it may be seen in figure 1, *c*, that often the processes appear on one side only, as was frequently observed in nuclei comparable to that of figure 1, *a*.

While these changes in the outgrowths are in progress, the main axis of the chromosome becomes more slender, and less readily stainable, but hardly to the degree which characterizes the outgrowths. Nonetheless, staining is the most difficult technical problem in the study of the lampbrush chromosomes: stain is extracted from the chromosomes almost as readily as from the nuclear background. A comparison of figure 1, *a* to *c*, shows that the main axis of the chromosomes is rapidly being spun out to a much more slender thread than the initial diplotene chromosome. Further, figure 1, *a* and *b*, show no indications of the doubleness of the chromosomes, whereas in figure 1, *c* the sister chromatids are clearly indicated in several places. At the chiasma in the upper part of the nucleus, the theoretically expected four strands are clearly

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Fig. 1, *a–c*, was drawn with the aid of a camera lucida, using an oil immersion lens. The proportions for fig. 1, *d–f*, were determined with a camera lucida and a 43 X lens, the details being filled in under oil immersion. All figures were drawn from *Amphiuma* oöcytes.

*a*) Cell 140$\mu$, nucleus 80$\mu$. Heidenhain's hematoxylin. Main axes are rather coarse, bristles quite frequent.

*b*) Cell 150$\mu$, nucleus 80$\mu$. Harris' hematoxylin. Main axes are becoming finer. The side branches are more diffuse, and a few have formed loops.

*c*) Cell 150$\mu$, nucleus 85$\mu$. Harris' hematoxylin. Side branches are clearly loops. Main axes show double, or even tetrad, character in some places.

*d*) Cell 300$\mu$, nucleus 140$\mu$. Heidenhain's hematoxylin. Note the fine caliber of both main axes and loops. Cross sections of chromomeres show 4 and 5 attached loops. Nucleoli are becoming very numerous.

*e*) Cell 510$\mu$, nucleus 187$\mu$. Harris' hematoxylin. Note the extremely fine diameter of all fibers, and the rosettes of loops around cross sections of chromomeres.

*f*) Cell 700$\mu$, nucleus 238$\mu$. Harris' hematoxylin. Stainability of the chromosomes is returning. Note the many nucleoli in contact with the chromosomes and the increased prominence of the nucleoli.

visible. There are other areas also in which the chromatids are clearly visible. A small, faintly staining nucleolus may be seen in contact with one of the chromosomes in figure 1, *c*.

The principal change seen in the chromosomes illustrated in figure 1, *d* (cell 300$\mu$, nucleus 140$\mu$) is the diminishing stainability of the chromosomes. Many of the chromosomes—main axes as well as outgrowths—are on the very verge of visibility. Other chromosomes, however, are still moderately visible, especially the main axes. In some of the side loops clear chromatin granules looking like chromomeres can be seen. In cross sections of such nuclei four to eight loops are seen, with a maximum at five or six. In this figure some nucleoli may also be seen in contact with the chromosomes.

With continued development all of the chromosomes reach the condition of the least visible ones of figure 1, *d*. Such a stage is illustrated in figure 1, *e* (cell 510$\mu$, nucleus 187$\mu$). Here all parts of the chromosomes can be made out only with the greatest difficulty. With the exception of rare fragments, the chromosomes have only the slightest affinity for the usual nuclear stains. It is surprising to find that such nuclei often stain better with Harris' than with Heidenhain's hematoxylin. Other than this general reduction of visibility almost to the vanishing point, the structure of the chromosomes appears unchanged from the preceding stage. This period is characterized by great growth in length of the main axes and processes of the chromosomes.

Coincident with, or slightly before, the first visible deposition of yolk, the chromosomes begin to regain their stainability. Lest this be regarded as a simple result of reconcentration of the chromosomes, it should be pointed out that the processes are not only still actively growing out, but they continue to for a long time. Apparently some physical or chemical change in the chromosomes is responsible for the increase in stainability. A nucleus at the beginning of this period is illustrated in figure 1, *f* (cell 700$\mu$, nucleus 238$\mu$). The return of stainability is first suggested in somewhat smaller eggs with nuclear diameters of about 215$\mu$. Although the illustrated nucleus is appreciably larger than this, only limited parts of the chromosomes, both axis and outgrowths, have become readily visible. Most remain very faintly staining and can be made out only with great difficulty. The morphological picture, however, differs only in one detail from preceding stages: many small nucleoli, some of them faintly staining, may be seen in contact with the chromosomes. A few of these are large. In addition to these there is the usual large number of nucleoli on or near the nuclear membrane, as has been true since the earliest stages. However, there is a progressive increase in size. The nucleoli shown in figure 1, *a*, average 1 to 2 micra in diameter, while those shown in figure 1, *c*, average 3 or 4 micra.

As deposition of yolk proceeds, the chromosomes continue to increase in stainability, until finally they reach a maximum in cells about the size of that illustrated in figure 2, *a* (cell 1020$\mu$, nucleus 225$\mu$). Here the outgrowths are quite definite, but the main axes are difficult to discern. One gets the impression that they are obscured by the many outgrowths. In a few sections everything remains hazy. The number of small nucleoli in contact with the chromosomes

has now become immense. The peripheral nucleoli include some as large as 6 or 7$\mu$ in diameter. Several points should be noted with respect to the side loops. First, the majority of them do appear as loops rather than single fibers, so that it seems justified to regard the appearance of single fibers as accidents of sectioning. Second, a chromomere-like structure is visible on some of the loops. Third, the loops vary considerably in size, the smallest extending only about 3$\mu$ from the main axis, whereas the largest illustrated extend 10 or 12$\mu$. Others (not illustrated) have been observed to fade into the nuclear background as much as 20$\mu$ away from the main axis. Lastly, it may be noted that several cross sections here show five loops radiating from a single point.

### Feulgen Preparations

Figure 2, *b*, the first in the Feulgen series, is included primarily for its bearing on stretching experiments. Usually the cytoplasm pulls away from the nucleus during fixation of these eggs, which are penetrated only slowly by most fixatives. This figure (cell 140$\mu$, nucleus 60$\mu$) shows a chromosome, part of which is stretched between the main part of the nucleus and a small part of the nuclear membrane which remained attached to the cytoplasm. Two segments of this chromosome were stretched more than 300 per cent. Reference to the figure will show that, while the chromomeres in the stretched regions are slightly compressed and elongated, most of the stretching has occurred in the fiber between the chromomeres. This fiber shows the Feulgen color quite clearly, so that evidently nucleic acid is not confined to the chromomeres, but is a component of the whole chromonema.

The pseudopod-like processes which are the earliest forerunners of the lampbrush structure are Feulgen positive, as indicated in figure 2, *c* (cell 70$\mu$, nucleus 50$\mu$). As reproduced in black and white, such a figure could be interchanged with a Heidenhain preparation of comparable stage, save for the more clear-cut picture given by the Feulgen reaction. The pseudopod-like processes show all of the characteristics described above for preparations stained by the traditional methods. At the ends of the large pair of chromosomes in this figure, the sister chromatids can be made out. At the chiasmata, however, they are completely synapsed, so that there is no visible evidence of the quadripartite structure which must be present.

At a somewhat later stage as represented in figure 2, *d* (cell 160$\mu$, nucleus 75$\mu$), the Feulgen positive material is restricted to the bases of most of the processes, only a few being stained at any considerable distance from the main axis of the chromosome. Thus the diminished affinity of these processes for the hematoxylins is paralleled by an actual loss of thymonucleic acid. Evidence will be presented below as to the chemical basis for this phenomenon. A striking feature of this stage is the frequent occurrence of large accumulations of thymonucleic acid along the main axis of the chromosomes, one of which is illustrated in this figure. In general, however, the main axes of the chromosomes are characterized by a steadily decreasing diameter.

If only the Feulgen positive elements are shown, then the chromosomes of figure 2, *e* (cell 220$\mu$, nucleus 90$\mu$) are substantially the same as those of figure

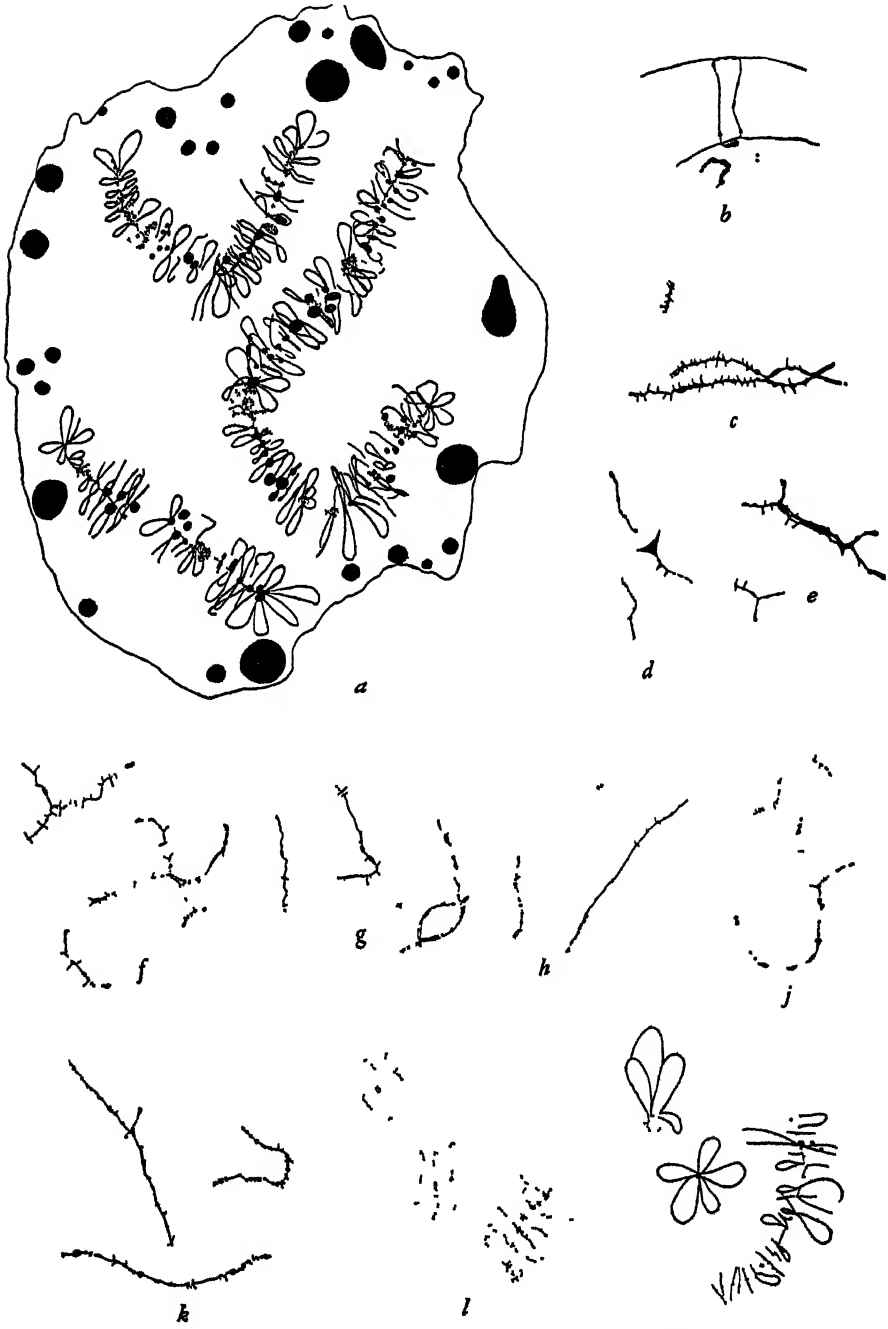


Figure 2. (For an explanation of this figure, see bottom of facing page.)

2, *d*. Processes, with a few exceptions, are positive only at their bases. The main axes are generally very fine, but include some large accumulations of chromatin. But unstained loops can be made out at many points, so that these chromosomes, if stained in Heidenhain's, would appear much like those illustrated in figure 1, *c*. These loops appear to contain no thymonucleic acid. The chromosomal segment illustrated between two chiasmata is noteworthy for two reasons. First, it is much more heavily laden with chromatin than are other parts figured, but nonetheless this nucleus contains no chromatin mass so large as that illustrated in figure 2, *d*. Second, although this segment generally appears single, its composite character is clear at several points, a maximum of three fibers being visible.

Throughout the remainder of their development, the lampbrush filaments remain Feulgen negative, except for their bases, which are frequently, but not always, positive. The main axes, however, remain positive, and become exceedingly slender, as may be seen in figure 2, *f* (cell 300$\mu$, nucleus 130$\mu$). Some extensive parts of these chromosomes appear as double rows of exceedingly small chromomeres. A row of eight of the chromomeres was found to be 7.2$\mu$ in length, hence, neglecting the spaces between chromomeres, each chromomere has a length of 0.9$\mu$. The width appears to be about half of the length. Thus these structures are very near the limit of visibility. These measurements were made with a Bausch and Lomb ocular micrometer which had been standardized against a Bausch and Lomb stage micrometer. Comparison with figure 2, *b*, or even with figure 2, *d*, will reëmphasize the extremely small dimensions of these chromomeres. Some larger chromomeres remain, however. In earlier figures, the sister chromatids have been so closely synapsed that they have

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Fig. 2, *b–m*, was drawn with the aid of a camera lucida, using an oil immersion lens. The proportions for fig. 2, *a*, were determined with a camera lucida and a 43 X lens, the details being filled in under oil immersion. All figures were drawn from *Amphiuma* oocytes.

*a*) Cell 1020$\mu$, nucleus 255$\mu$. Heidenhain's hematoxylin. This figure represents the maximum development of the lampbrush chromosomes.

*b*) Cell 140$\mu$, nucleus 60$\mu$. Feulgen. Note the great stretching of the chromonema, and slight deformation of the chromomeres, in the stretched chromosome.

*c*) Cell 70$\mu$, nucleus 50$\mu$. Feulgen. The bristles are Feulgen positive. Sister chromatids can be made out at some points.

*d*) Cell 160$\mu$, nucleus 75$\mu$. Feulgen. Note the fine diameter of the main axes. Only the bases of bristles are positive. One chromosome includes a very large accumulation of thymonucleic acid.

*e*) Cell 220$\mu$, nucleus 90$\mu$. Feulgen. Characteristics are much as in figure *d*, but the chromatids are visible between the two chiasmata.

*f*) Cell 330$\mu$, nucleus 130$\mu$. Feulgen. Chromatids are clearly visible as rows of very fine chromomeres, on some of which fine bristles may be seen.

*g*) Cell 400$\mu$, nucleus 150$\mu$. Feulgen. Note the two chiasmata with a nearly complete internode between them.

*h*) Cell 650$\mu$, nucleus 200$\mu$. Feulgen. Chromosomes are not substantially different from those of figure *f*.

*i*) Cell 720$\mu$ nucleus 230$\mu$. Feulgen. Chromosomes are much like those of figure 2, *f*, *g*, and *h*, although a comparable cell stained in hematoxylin would show a return of stainability.

*j*) Cell 850$\mu$, nucleus 270$\mu$. Feulgen. The main axis is now somewhat thicker, but only the bases of the loops are Feulgen positive.

*k*) Cell 850$\mu$, nucleus 300$\mu$. Feulgen. Note the chiasma, in which 3 of the expected 4 strands are visible.

*l*) Cell 1020$\mu$, nucleus 280$\mu$. Unna. Loops are faintly stained, but the main axes are negative.

*m*) Cell 1300$\mu$, nucleus 315$\mu$. Unna. Loops are definitely stained. Main axes may be stained at some points, but very faintly.

not been resolvable except for a few short segments, but here they appear clearly, even widely, separate for long distances. Internodal parts of the homologues are now so widely separated that one frequently sees in the sections only one member of the pair. Their actual status as synapsed homologues is made apparent only by the occasional appearance of chiasmata.

Figure 2, *g* (cell 400$\mu$, nucleus 150$\mu$) does not show any substantial change from the condition just described. It includes, however, two chiasmata with a very nearly complete internode between them. At one of these chiasmata, all four threads are clearly visible, and their arrangement at the chiasma is as expected on the basis of the chiasma-type theory. The difficulties of visibility which Heidenhain or other traditional preparations present at this stage are not experienced with Feulgen preparations. The strands become exceedingly fine, but the main axes give a definite Feulgen reaction and contrast clearly with the background at all stages.

As nuclei of 200$\mu$ are substantially similar to those of 150$\mu$ in Heidenhain preparations, it is not surprising that the chromosomes of figure 2, *h* (cell 650$\mu$, nucleus 200$\mu$) show no marked differences from those of figure 2, *g*, in the Feulgen series. But it is surprising to find this true also of figure 2, *i* (cell 720$\mu$, nucleus 230$\mu$), for such a nucleus would show a definite return to stainability in a hematoxylin preparation. Nonetheless, the Feulgen stained chromosomes do not look essentially different from those of the much smaller nuclei represented in figure 2, *f* to *h*.

As hematoxylin preparations show strongly staining chromosomes with large clear loops in nuclei of cells with a well-advanced deposit of yolk, it is of interest to note that the Feulgen picture of such nuclei is far less changed from preceding stages than might have been expected. Figure 2, *j* (cell 850$\mu$, nucleus 270$\mu$) shows that the main axis is considerably coarser than in preceding stages, but hardly to the degree which might be expected on the basis of hematoxylin preparations. Further, although the side loops are striking in hematoxylin preparations at this stage (see fig. 2, *a*), only the bases of the loops, and perhaps not of all loops, are Feulgen positive. These same conditions still apply to the chromosomes illustrated in figure 2, *k* (cell 850$\mu$, nucleus 300$\mu$). None of these chromosomes is coarse enough to be compared to the early stages in their development, as illustrated, for example, by figure 2, *c*. One very slender chromosome shows a chiasma with three of the expected four strands visible, although at other points along the length of this chromosome there is no visible evidence of doubleness. The other chromosomes illustrated in this figure are somewhat thicker, but they include parts in which sister chromatids may be seen side by side. Loops are still negative except at their bases.

Needless to say, nucleoli are negative in all Feulgen slides. The Feulgen positive granules which Painter and Taylor (1942) have reported to be the centers of organization for nucleoli in eggs of *Bufo* were not observed in eggs of *Amphiuma*.

### Unna Preparations

Brachet (1940*b*) has shown that the pyronine component of Unna's pyronine-methyl green mixture may be regarded as specific for ribonucleic acid. This stain is less satisfactory than the Feulgen reaction, not only because its chemical basis is unknown, but also because it is a pale stain which does not stand out sharply on slides. A series of slides was stained with this mixture in order to ascertain whether ribonucleic acid enters into the composition of the chromosomes.

Very early oöcytes (cells of about 80$\mu$ with nuclei of about 50$\mu$) are completely negative to Unna's stain. In a later stage (cell 140$\mu$, nucleus 60$\mu$), the cytoplasm is positive, but the nucleus is negative. If any nucleoli are present, however, they are positive. As the cell grows, this picture is changed only by the increase in size and numbers of nucleoli, all of which are positive at all stages. The chromosomes remain completely negative until very late in their development. Figure 2, *l* (cell 1020$\mu$, nucleus 280$\mu$) shows a group of chromosomes which are so faintly positive that they can be drawn only with great difficulty. Somewhat earlier nuclei give one the impression that stained material is present, but so faint that drawing is impossible. The stain is largely, perhaps entirely, confined to the side loops.

Figure 2, *m* (cell 1300$\mu$, nucleus 315$\mu$) shows chromosomes which are plainly stained with pyronine. Here again the side loops are the prominently stained parts. Some parts of the main axis may be stained, but they are not clear—less so on the slides than in the figure. Many small nucleoli may be seen in contact with the chromosomes.

### Observations on Nuclease-Digested Tissue

*Feulgen preparations.*—The most important test on nuclease-digested tissue is staining by the Feulgen reaction. The results depend on the duration of enzyme action. The Feulgen reaction is usually visibly paler on slides digested for 24 hours, but much longer periods, usually 48 hours or more, are required for the complete removal of thymonucleic acid, as evidenced by a negative Fuelgen reaction. In these experiments, digestion time ranged from a few hours up to 96. The most resistant stages are those early ones in which large masses of chromatin occur. Thus only prolonged digestion, over 60 hours, will completely remove the thymonucleic acid from nuclei whose diameters are under 90$\mu$. But the larger nuclei often are completely Feulgen negative after 48 hours. By contrast, the very dense nuclei of red blood cells never appear completely negative, even in the slides digested for as long as 96 hours.

Incompletely digested slides differ from undigested controls only in the reduced intensity of stain. Every structure described above for undigested Feulgen preparations appears in these partially digested slides unchanged except for the reduced intensity of staining. Even in slides the Feulgen reaction of which is on the verge of disappearing there is no sign of alteration of the morphology of the chromosomes. In one very young cell (cell 100$\mu$, nucleus 60$\mu$), however, the completely negative processes no longer had the

appearance of pseudopodia, but rather of small loops. The theoretical significance of this will be discussed below.

*Unna preparations.*—In order to trace the history of the ribonucleic acid in nuclease-digested slides, a series was stained with Unna's mixture. As all slides were completely negative, it was concluded that the nuclease used was not pure, but contained a significant amount of ribonuclease as well as thymonuclease. If this assumption is correct, then these results may be regarded as a further indication that Unna's mixture is specific for ribonucleic acid.

*Hematoxylin preparations.*—Attempts were made to stain the chromosomes by various chemical tests for proteins, such as the ninhydrin test, in the hope that these methods would demonstrate the protein skeleton of the chromosomes, especially in nuclease-digested preparations, as has been done by Kodani (1942) and by Mazia and Jaeger (1939). But these tests failed both on digested slides and on untreated slides. The failure was attributed to the extremely fine dimensions of the lampbrush chromosomes.

Because of the failure of the protein tests, it was hoped that a continuous basis for the nuclease-digested chromosomes could nonetheless be demonstrated by the use of iron hematoxylin, as Kodani (1942) has done for the nuclease-digested so-called lampbrush chromosomes of *Drosophila*. In order to be sure that staining did not depend on residual thymonucleic acid, all slides for this experiment were digested for periods in excess of 72 hours.

All of the structures described above in the section on undigested hematoxylin preparations were again visible, although the stain was consistently fainter. This applies equally to the main axis of the chromosomes at all stages, to the pseudopod-like processes of the earliest stages and to the loops of the later stages. During the stage of minimum visibility (nuclei with diameters ranging from 100$\mu$ to about 210$\mu$) when the chromosomes can be made out only with great difficulty in undigested preparations, they are, of course, exceedingly faint in these nuclease-digested slides. But they can always be made out if optimum optical conditions are used, and occasional nuclei show them well.

It thus appears that in the lampbrush chromosomes of *Amphiuma* as well as in the salivary gland chromosomes of *Drosophila* (Kodani, 1942, Mazia and Jaeger, 1939) the morphological continuity of the chromosomes is not dependent on nucleic acid but on some other substance which the authors cited have shown to be protein in the case of the salivary gland chromosomes. In the absence of evidence to the contrary, one would assume that the continuity of the lampbrush chromosomes also depends on proteins, and evidence will be presented below that this assumption is correct.

### Observations on Pepsin-Digested Tissue

*Hematoxylin preparations.*—Slides were treated with pepsin for periods varying from 4 to 24 hours. The results differed according to the stage of development of the eggs, but one result was consistent at all stages: visibility was markedly improved, probably because of removal by the enzyme of stainable materials other than the chromosomes.

In the earliest stages (nuclei up to about 70$\mu$) the chromosomes appear as

heavy, smooth bands, more suggestive of mitotic chromosomes than of incipient lampbrush chromosomes. It seems probable, however, that the processes have not been digested off as one would expect, but have instead shrunken. The reasons for this view will be discussed below.

In older nuclei (diameters of 70$\mu$ to 100$\mu$) the main axes still appear as prominent bands, but processes are now visible. They are, however, smaller and fewer in number than the processes of undigested chromosomes. This is what one would expect, if, as suggested above, the processes are being shrunken but not digested off. Once definite loops are formed, they seem to remain visible throughout enzyme treatment—in other words, they are too large to blend into the main axis when they shrink under the influence of pepsin.

During the stage of minimum visibility everything appears as in undigested preparations, except that the chromosomes stand out more clearly in the pepsin-digested preparations. Jörgensen (1913) and Duryee (1941) have reported that the side branches of lampbrush chromosomes are removed by pepsin. Some slides at this stage suggest that this may have happened, but wherever the chromosomes are clearly stained, the side branches seem to have remained intact. In the later stages (nuclei with diameters of 250$\mu$ or more) the loops are strikingly more prominent in pepsin-digested slides than in any other type of preparation. It is plain that there is no digestion of loops here. One gets the impression that the threads making up the loops are somewhat thicker than those of untreated slides, as though there had been some shortening and thickening. Because of the extreme fineness of the threads, however, one cannot check this impression by accurate measurement. Not infrequently one sees a loop which appears to be double, as though it consisted of synapsed threads. This appearance might well be produced in other ways, however. For example, if the loop should be made up of a single finely-coiled thread, then the nodes of the coil might well appear as such double rows. Shrinkage would be expected to accentuate such a picture.

*Feulgen preparations.*—As in hematoxylin preparations the processes are absent or few and reduced in the earlier stages in Feulgen preparations. Soon, however, the pepsin-digested Feulgen preparations become indistinguishable from the undigested Feulgen preparations. At all stages, processes are less frequent, indicating that the thymonucleic acid-containing material at the bases of the loops has been sufficiently contracted that it no longer shows up distinctly from the main axis of the chromosomes. Sections in which the chromatids show up separately are less frequent than in undigested slides, and this may also be the result of shrinkage. In the largest nuclei no difference whatever can be detected between the Feulgen preparations of pepsin-digested and control slides.

### Observations on Trypsin-Digested Slides

*Hematoxylin preparations.*—Slides were digested in trypsin for periods varying from 4 to 12 hours. The results vary from minor destruction after 4 hours to complete destruction of the sections after 12 hours digestion. For any

period of digestion the youngest eggs are the ones most badly damaged, and in slides digested more than 6 hours ova under 100$\mu$ in diameter are rarely seen.

Both loops and main axes are destroyed by trypsin. Although the loops disappear while the main axes are still clear, there is no evidence that the loops are more susceptible to digestion by trypsin than are the main axes of the chromosomes. Loops are still visible, though incomplete and less frequent, long after the axes show evidence of having been attacked. Thus it appears that the loops disappear sooner than the axes simply because their dimensions are finer.

It should be noted that the cytoplasm is usually extensively digested before the nuclei show the effects of trypsin digestion; and that the nucleoplasm is badly damaged while the main axes of the chromosomes appear fairly well intact. Thus it appears that, while the chromosomes are in time completely destroyed by trypsin digestion, they are rather resistant as compared to other structures of the cell. In this respect, Koltzoff (1938) may well have been right when he referred to nucleic acid as a protective coating on the chromosomes, for the more basophilic parts of the cytoplasm also resist trypsin digestion longer than do the other parts. As cytoplasmic basophilia is probably caused by ribonucleic acid, as shown by Unna staining, it seems probable that protection of proteins with which they are conjugated against trypsin digestion is a general property of nucleic acid.

*Feulgen preparations.*—The Feulgen picture in trypsin-digested material parallels the hematoxylin picture in much the same way that Feulgen preparations of undigested chromosomes parallel hematoxylin preparations. With short periods of digestion the main axes are intact, and a somewhat reduced number of barbs (i.e., bases of loops) is visible. With continued digestion the main axes are broken into shorter pieces, become reduced in number, barbs disapper, and finally the chromosomes disappear altogether.

One significant difference between pepsin-Feulgen and trypsin-Feulgen preparations should be noted. At all stages in pepsin-digested tissue, Feulgen staining is strictly limited to the chromosomes. After only 4 hours of digestion with trypsin, however, a fairly intense staining occurs throughout the nucleus, although the chromosomes are much more intensely stained than the nucleoplasm. It appears, then, that the nucleic acid is bound to the chromosome by a protein which is susceptible to trypsin digestion, but not to pepsin digestion. In the late stages of trypsin digestion the nucleoplasm becomes Feulgen negative. It is not clear whether this means that the nucleic acid diffuses from the section very slowly, or that it is precipitated on nucleoplasm proteins which are slowly digested away.

## OBSERVATIONS ON *SQUALUS SUCKLEYI*

*Hematoxylin preparations.*—As in *Amphiuma*, hematoxylin preparations of ova of *Squalus* show the classic lampbrush picture. At all stages, however, the details are less clear so that interpretation is greatly facilitated by comparison with *Amphiuma*.

The chromosomes of the smallest cell observed (cell 110$\mu$ nucleus 45$\mu$) are

quite similar to those of figure 1, *a*. The barbs, however, are more numerous and coarser. Nucleoli are already prominent, some being as large as those of the most advanced *Amphiuma* nuclei. The cytoplasm is strongly basophilic, and remains so throughout the period of development of the lampbrush chromosomes.

These barbs quickly assume the more diffuse appearance of the pseudopod-like processes illustrated in figure 1, *b*, for in a 50$\mu$ nucleus (cell 200$\mu$) most of the processes have this form. The loss of stainability which accompanies this change is less marked than that which occurs in *Amphiuma*, nonetheless the details of structure are less readily observed in *Squalus* than in *Amphiuma*. The individual pseudopodia are quite close together, so that they can be resolved only with an oil immersion lens. Even this is made difficult by the chromatic nucleoplasm. This picture is not substantially changed until the nucleus reaches a diameter of about 85$\mu$ (cell 340$\mu$), when many of the pseudopodia appear to break through in the center, leaving small loops. Such cells are similar to figure 1, *c*, but their appearance is decidedly coarser. A few loops may appear in younger nuclei (as small as 70$\mu$), while in some cases loops will not appear until the nuclear diameter is about 90$\mu$. Meanwhile, nucleoli have reached a degree of prominence found only in the largest nuclei of *Amphiuma*.

In nuclei of 105$\mu$ (cell 470$\mu$) all of the processes appear to have reached the loop stage, and further development is a matter of growth, involving very little morphological change. As expected, this process involves a loss of stainability of the chromosomes until they reach a minimum at which the chromosomes have scarcely more affinity for basophilic stains than does the nucleoplasm. However, this loss of stainability is much slower than it is in *Amphiuma*, for the chromosomes of nuclei up to 120$\mu$ are still fairly readily visible, and a similar condition was observed in one nucleus of 150$\mu$. Figure 1, *e* represents quite well the chromosomal condition of *Squalus* nuclei ranging from 130$\mu$ up to 230$\mu$ (cells 650$\mu$ to 2200$\mu$). Throughout this range, while the lampbrush loops are becoming ever longer and finer, the chromosomes are exceedingly faint, but the typical structure, as illustrated in figure 1, *e*, can always be made out by careful observation and use of optimum optical conditions. Throughout this period, the nucleoli remain more numerous and larger than those of *Amphiuma*.

The first visible deposition of yolk occurs when the nuclear diameter is about 240$\mu$ and the cell diameter is about 2200$\mu$. Unlike *Amphiuma*, this was not accompanied by any striking increase in stainability of the chromosomes in *Squalus*. Rather, there was a gradual increase which did not go beyond the condition indicated in figure 1, *f*, in any cell observed. The largest cell observed had a total diameter of about 3000$\mu$ and a nuclear diameter of about 270$\mu$. The deposit of yolk is extensive even in considerably smaller cells (cell 2700$\mu$, nucleus 250$\mu$). As previous studies on the lampbrush chromosomes of elasmobranchs (Rückert, Maréchal) have shown an increase in stainability comparable to that which occurs in *Amphiuma*, it seems probable that similar results would have been obtained with *Squalus* had the material studied in-

cluded larger eggs. It is surprising that the nucleoli are less numerous in these very large eggs than in the preceding stage. They are now comparable in quantity to the nucleoli in *Amphiuma* nuclei of the same stage.

*Feulgen and Unna preparations.*—Feulgen staining was decidedly less satisfactory with *Squalus* than with *Amphiuma*. This may be because of the fixatives used (Gilson's and Karpechenko's), for the ovaries were not fixed especially for use with the Feulgen stain. Nonetheless, the Feulgen preparations support the *Amphiuma* series to some extent. In nuclei up to 60$\mu$ in diameter the pseudopodia as well as the main axes are positive. In nuclei over 120$\mu$ in diameter even the main axis is negative and remains so throughout the period studied. It seems improbable that this represents the actual condition of the chromosomes. It may be that the chromosomes are spun out into such fine threads that the reacting thymonucleic acid is below the limit of visibility. More probably sections from material fixed in Bouin's fluid will reveal the main axes.

Unna's stain gave much the results expected on the basis of the *Amphiuma* slides. In the youngest cells studied, the cytoplasm and the nucleoli are strongly positive. The nucleoli remain so throughout the development of the egg. The intensity of the staining of the cytoplasm declines in the older eggs, perhaps because the rate of production of ribonucleic acid lags behind the growth of the cytoplasm; or perhaps because the ribonucleic acid enters into cytoplasmic reactions which render it unstainable. No sign of staining of the chromosomes was seen in even the most advanced nuclei studied. This may mean that the loops of chromosomes of *Squalus* contain no ribonucleic acid or it may mean that ribonucleic acid, although present, is too finely drawn out to be observed with this very weak stain. Especially when considered in relation to the positive results in large eggs of *Amphiuma,* the latter possibility seems the more probable.

*Digestion experiments.*—The results of nuclease digestion agree with those obtained from *Amphiuma*. The chromosomes of all stages are completely Feulgen negative, but they are faintly stainable with hematoxylin. Both loops and main axes can be seen to remain intact. All parts are completely destroyed by trypsin.

## DISCUSSION

### Morphological Considerations

Maréchal (1906) regarded the question of the persistence or nonpersistence of the chromosomes as of prime importance. This question may now be regarded as decisively settled in favor of persistence, as all subsequent studies have supported Maréchal in this respect.

However, it is now possible to extend Maréchal's conclusions. Because the chromosomes remain visible, though very faintly so, during the growth phase of the lampbrush chromosomes, he was convinced of their morphological continuity or individuality. But because of the extremely low stainability of the chromosomes during this period, he thought that the chromatin must have been destroyed, to be formed anew during the phase of reconcentration of the chromosomes. It is now possible to extend to the chromatin the concept

of continuity throughout the chromosomal cycle, at least in the main axis of the chromosomes. Brachet (1940*a*) and Painter and Taylor (1942) have previously demonstrated the presence of thymonucleic acid in the main axes of lampbrush chromosomes by means of the Feulgen reaction, which had not been discovered in the time of Maréchal. In the present study the thymonucleic acid has been traced, by means of the Feulgen reaction together with nuclease, from the earliest lampbrush chromosomes up to the beginning of the recondensation of the chromosomes. The main axis has been shown to contain thymonucleic acid at all stages, although the side branches contain it only at the earliest, or pseudopod-like stage. Thus the principle of the persistence of the chromosomes extends not only to an achromatic basis of the chromosomes but to the chromatin as well.

As the side loops are the characteristic structures of the lampbrush chromosomes, their mode of origin and their relationship to the ordinary chromosome are of especial interest. The first step in their formation in the organisms studied appears to be the extension of pseudopod-like processes from the chromomeres of the diplotene chromosomes (figure 1, *a* and *b*). This may be designated as stage I. These pseudopodia at first contain thymonucleic acid as shown by a positive Feulgen reaction which becomes negative after nuclease treatment. This thymonucleic acid soon disappears. As the pseudopodia extend out from the main axis of the chromosome, they begin to appear as loops, as though their centers had been broken through, like soap films on wire loops (figure 1, *b* and *c*). When the majority of the pseudopodia have thus become small loops, the lampbrush structure may be regarded as established, and the chromosomes now begin their great expansion which constitutes the second phase (stage II) of their development (figure 1, *c–e*). During most of this phase, the chromatin is finely dispersed, and so the chromosomes are difficult to stain. This is the critical period in which authors of the Schultze school believed that the chromosomes were destroyed. Following this phase, and coincident with the deposition of yolk in the cytoplasm, the chromosomes regain their stainability (stage III), probably because of the presence of increasing concentrations of ribonucleic acid in the loops (figure 1, *f*, figure 2, *a*, *l*, and *m*). The main axis at this stage is obscured in hematoxylin preparations by optical overlapping of the central parts of the loops (figure 2, *a*). Lastly, the chromosomes undergo a reconcentration to form the chromosomes of the first meiotic spindle, a phase not included in the present study.

The above interpretation is at variance with those of some other authors. Koltzoff (1938) regarded the loops as being formed by maximal extension of some members of a bundle of chromonemata, these synapsing at homologous points with their (molecularly) less extended sisters. It seems improbable that he would have held this opinion had he succeeded in staining the lampbrush chromosomes with the Feulgen reagent, for in Feulgen-stained slides the main axis appears as a straight line or chain of chromomeres, with the faint, unstained loops extending laterally from the main axis at regular intervals. To explain these facts under Koltzoff's theory, it would be necessary to make the improbable assumption that those parts of the accessory chromonemata

which are synapsed with the main axis contain thymonucleic acid while the looped parts do not; or one would have to make the even more improbable assumption that the whole accessory chromonema is unstainable, but visible nonetheless only in those parts which are not synapsed with the main axis. Further, the main axis, which stands out very clearly in Feulgen preparations, is very little thicker than the loops, and this does not suggest a very different order of molecular unfolding.

According to Ris (1945), the lampbrush chromosomes are simply elongated diplotene chromosomes in which the four chromatids have formed major coils and separated from one another laterally. The central overlapping of the gyres gives the impression of a main axis with chromomeres. This is also difficult to harmonize with the results of Feulgen staining, for it does not account for the very clear, precise main axis which is seen in such preparations. Further, it is difficult to believe that only the overlapping parts in such a system would be Feulgen positive, all of the rest being Feulgen negative. Also, Ris's conception of the structure of the lampbrush chromosomes requires that cross sections should always show four loops. Actually, such figures are seen at times, but much more frequently five loops are seen, and some cross sections show six, seven, or even eight loops, all of which would be impossible if Ris's conception were correct.

The interpretation of Painter and Taylor (1942) agrees with the present interpretation in regarding the loops as point outgrowths. But, because they obtained negative results on the loops both with Feulgen and Unna stains, these authors regard them simply as lateral expansions of "matrix." Presumably, matrix means inactive material. In view of the evidence of intense metabolism (formation of nucleoli) in these loops, however, such an interpretation is hardly tenable. Further, the present study indicates that the loops do contain ribonucleic acid (positive Unna stain during stage III—figure 2, *l* and *m*).

There is perhaps only an apparent contradiction between the present study and that of Duryee (1941). The mechanism of loop formation which he describes—division of a single chromiole to form two which remain connected by a bar which continues to grow and thus forms a loop—is substantially the same as that described in this paper. These modes of loop formation become identical if stainable substance fills in the smallest loops of Duryee, thus giving them a pseudopodial appearance. The differences between his description and the present one may well be only the differences between fresh and fixed tissue. There is, however, a further difficulty. Duryee's description would appear to allow only one loop, or at the most two, to a chromomere, whereas five loops is the number most frequently observed in cross sections of the chromosomes. It seems probable, however, that a comparison of hematoxylin and Feulgen preparations, together with Kodani's work on the salivary-gland chromosomes, may resolve this apparent conflict. During stage II, when the chromosomes have their minimum visibility, Feulgen preparations show that the main axis is made up of exceedingly small chromomeres, which are very near the limit of visibility (figure 2, *f*, *g*, and *i*). Hematoxylin preparations fail to reveal

these granules, which are paired and which may be the chromioles of Duryee. In stage III, when the chromosomes are more readily stained by hematoxylin, a coarser structure of the main axis is indicated by both hematoxylin and by the Feulgen reaction (figure 2, *a* and *h*). Now Kodani (1942) has shown that the bands of the salivary gland chromosomes are essentially coiled segments of the chromonemata to which are attached nucleic acid-containing bulbs, usually a series of five bulbs to a homologue, or ten to a band. If a short segment of the lampbrush chromosome, including five pairs of the chromioles of Duryee, were to coil very tightly, they would collectively give the appearance of the chromomeres of stage III, and the attached loops would form the rosette so frequently seen in cross sections of lampbrush chromosomes.

One more question must be considered with respect to the loops: are they latent in the normal chromosomes as Rückert (1892) suspected or are they without counterpart in normal chromosomes? A decisive answer to this question must come from a study of the normal chromosomes themselves, perhaps by the methods of Kodani. Meanwhile, there are some data which indicate that the lampbrush structure is latent in the normal chromosomes. Thus, the pachytene and diplotene chromosomes of animals generally and of Orthoptera especially acquire a fuzzy appearance which may well be analogous to the pseudopod formation of stage I lampbrush chromosomes. Nor is the ability of chromomeres to put out pseudopod-like processes confined to meiotic chromosomes, for Wilson (1925) cites many examples in which mitotic chromosomes "send forth branches" in the telophase reconstruction. In this connection it is important to note that Rückert regarded the lampbrush chromosomes as substitutes for the resting condition to permit the intense metabolism of the growing egg. Lastly, Kodani has demonstrated that the chromatic bulbs of salivary-gland chromosomes tend to flow out like pseudopodia under the influence of alkali, or various other reagents. Thus it appears that the ability of chromomeres to put out pseudopodia is not confined to incipient lampbrush chromosomes but is a general characteristic, and so it seems probable that the lampbrush loop is latent in the normal chromomere.

Although no one has doubted the tetrad character of the lampbrush chromosomes, previous studies have not yielded visible evidence of it. Duryee (1941) refers to each chromosome as "a single plastic cylinder," although his radiation experiments gave evidence of a latent cleavage plane within this cylinder. All previous students of the lampbrush chromosomes have observed chiasmata, and so it is clear that the homologous chromosomes are still associated, although they separate as widely as possible between chiasmata (figures 1, *c*, 2, *f* and *g*). But previous studies have failed to show the two sister chromatids which it was necessary to assume made up each homologue. In the present study, however, Feulgen preparations, especially of stage II cells, show the separate chromatids as parallel rows of extremely fine chromomeres, the strand between the chromomeres also appearing chromatic at times. This is shown especially well in figure 2, *c*, *f*, *g*, and *i*. A comparative study of the figures shows that the sister chromatids are most easily visible in those chromosomes in which the chromatin is most finely dispersed. In some instances

(figure 2, *f* and *g*) the chromatids are widely separated, relative to their own width. These facts suggest that there may be an inverse relationship between the degree of dispersion of the chromatin and the closeness of synapsis: the more finely dispersed the chromatin, the less closely synapsed the chromosomes. The extreme fineness of these chromosomes suggests that they are maximally extended, but the stretching experiments of Duryee prove that they are not.

### Lampbrush Chromosomes and Salivary Gland Chromosomes

In their paper of 1942 Goldschmidt and Kodani pointed out the close structural similarity between the naturally occurring lampbrush chromosomes and those produced by Kodani's methods from the salivary gland chromosomes. They observed that in both there is a chromonema to which are attached loops (naturally occurring lampbrush chromosomes) or chromatic hairs (salivary gland chromosomes). These structures are of the same order of magnitude. Digestion experiments with nuclease and trypsin showed that the chromatic hairs had a protein basis as well as a nucleic acid component, thus extending their resemblance to the loops of natural lampbrush chromosomes, which were then supposed to be entirely achromatic.

It is now possible to extend this comparison. Kodani (1942) has demonstrated that, in each band of the salivary gland chromosomes, one homologue (with its two sister chromatids) is coiled in one direction, while the other homologue is coiled in the other direction, each occupying half of the cross section of the chromosome. Each coiled homologue bears a series (usually five) of paired but closely synapsed chromatic bulbs which are radially arranged. Thus each chromatic band is made up of an aster usually of ten pairs of chromatic bulbs, together with interstitial chromatin. Double bands may be formed by failure of synapsis between the chromatic bulbs of sister chromatids.

As pointed out above, the data reported in this study can be harmonized with Duryee's if one assumes that short segments of loop-bearing chromomeres are tightly coiled to form larger secondary chromomeres, with the attached loops forming a rosette. While the number of loops in each such rosette varies, five is the most frequent number. It is curious that this is also the number of pairs of chromatic bulbs which Kodani found most frequently attached to a single chromosome coil in the salivary gland chromosomes. Thus each part of Kodani's salivary lampbrush chromosomes has a very suggestive counterpart in natural lampbrush chromosomes: chromonemata, coiling, and loops or hairs. Nor is the contrast between chromatic hairs and achromatic loops so sharp as it formerly appeared, for experiments with Unna's stain indicate the presence of ribonucleic acid in the loops of stage III cells (figure 2, *l* and *m*). If the space between the several loops of each rosette were to be filled in with chromatin, and if the chromonemata between each rosette were to swell with "matrix" to reach the diameter of the rosettes, then we would have produced the structure of a salivary gland chromosome from a lampbrush chromosome.

Thus one might safely compare these two types of chromosomes, although

one is a natural occurrence and the other is an experimental artifact. The most detailed comparison shows that the artifact is based on structural potencies which are the same as those naturally realized in the real lampbrush chromosomes. In both, the highly modified chromosomes arise as an adaptation to the intensive metabolism of the cells concerned. All chromosomes show a tremendous increase in surface area during the greatest cell activity, the chromatin dispersal of "resting" nuclei. All chemical and cytological investigations have yielded substantially the same results for chromosomes of whatever source. It may well be then, that the nature of all chromosomes is such that adaptation to the most intense metabolism can only result in something similar to the lampbrush structure of vertebrate eggs or of salivary gland chromosomes. It must be emphasized that this is conjecture.

## Size Relations

It is not possible to compare the mitotic and lampbrush chromosomes without being impressed by the tremendous increase in size of the latter. Rückert attempted a quantitative estimation of the increase. He calculated the volume of a typical metaphase chromosome of *Pristiurus* and also the total volume occupied by a typical lampbrush chromosome, including, however, the nucleoplasm within its meshes. On this basis he concluded that the volume increase was on the order of 12,000 to 14,000 times.

If the assumption (to be discussed below) is correct that lampbrush formation is primarily an adaptation to get more extensive surface for reactions, then a comparison of surface areas would be more significant than a comparison of volumes. An attempt has been made to make such a comparison of surface areas of mitotic and lampbrush chromosomes of *Amphiuma*. A typical mitotic chromosome was found to have a diameter of $2\mu$, and a length of $18\mu$. This gives a surface area of approximately 120 square micra. Calculations for the lampbrush chromosomes are based upon chromosomes at the height of their development (stage III). The main axes of such chromosomes were estimated to average perhaps $300\mu$ in length and about 1 micron in diameter, giving a surface area of 942.5 square micra.

To determine the surface areas of the side loops, several representative segments of chromosome were selected and the length and width of each loop measured. Average length of loops was found to be $9\mu$, while the average width was $2.5\mu$. The length of the thread in the average loop was then calculated as the circumference of a circle with a diameter midway between these two values. This gives a circumference of 22 micra. The threads making up the loops are too fine to permit direct measurement of diameter, but were estimated to vary from $0.2\mu$ to $0.5\mu$. If a diameter of $0.3\mu$ is assumed, then the average circumference of the thread is $0.94\mu$. Thus the surface area of the average loop is 20.7 square micra.

The number of loops per chromomere, as seen in cross sections, varies from 4 to 8. Much the most frequent number, however, is 5. The loop-bearing chromomeres are spaced at intervals of $2\mu$ or less, and so the average chromosome should bear about 150 groups of 5 loops each. This gives a total

surface area for the loops of 15,525 square micra and a total for the chromosome of 16,467 square micra. Dividing the total surface area of the lampbrush chromosome by that of the mitotic chromosome gives an increase of 137.2 fold or 13,720 per cent.

Another aspect of size relations is the nucleoplasmic ratio. In order to determine the nucleoplasmic ratio for cells with nuclei of a particular size, for example 50$\mu$ in diameter, maximum diameters of all cells with this size nucleus were recorded and averaged. Volumes of the nuclei and of the cells as a whole were then computed on the basis of the assumption that each was a sphere. The nucleoplasmic ratio was then obtained by dividing the nuclear volume by cell volume minus the nuclear volume. Nuclear diameters chosen

TABLE 1

COMPARISON OF N/P RATIOS OF *Amphiuma* AND *Squalus*

| Nuclear diameter | | Cell diameter | | N/P ratio | |
|---|---|---|---|---|---|
| Amphiuma | Squalus | Amphiuma | Squalus | Amphiuma | Squalus |
| 50$\mu$ | 50$\mu$ | 86$\mu$ | 185$\mu$ | 1/4 | 1/50 |
| 75$\mu$ | | 144$\mu$ | | 1/6 | |
| 100$\mu$ | 100$\mu$ | 220$\mu$ | 405$\mu$ | 1/10 | 1/65 |
| 120$\mu$ | | 330$\mu$ | | 1/20 | |
| 200$\mu$ | 200$\mu$ | 650$\mu$ | 1000$\mu$ | 1/50 | 1/125 |
| | 270$\mu$ | | 3000$\mu$ | | 1/1400 |
| 300$\mu$ | | 1470$\mu$ | | 1/120 | |

for determination of nucleoplasmic ratio are the following: 50$\mu$, 75$\mu$, 100$\mu$, 120$\mu$, 200$\mu$, and 300$\mu$. The results are summarized in table 1. The first three horizontal lines show that little change occurs in the nucleoplasmic ratio during the first, or pseudopodial stage, when the lampbrush form of the chromosomes is in its earliest beginnings. The ratio is about 1 to 4 when the pseudopodia are first detectable and is about 1 to 10 by the time that the lampbrush form is definitely established.

The following four lines of table 1 show that the cell volume increases at a much greater rate than does nuclear volume once the lampbrush chromosomes have been established and have begun their own great growth. Thus, when the nucleus of *Amphiuma* has a diameter of 120$\mu$, the nucleoplasmic ratio has become 1 to 20; when the nuclear diameter has become 200$\mu$, the ratio has become 1 to 50; and when the nuclear diameter is 300$\mu$, the ratio has reached 1 to 120.

Some nucleoplasmic ratios were also calculated for *Squalus*, and these have been included in table 1 for purposes of comparison. The same trends occur in the ova of both organisms. A given size of nucleus, however, always controls a much greater amount of cytoplasm in *Squalus* than in *Amphiuma*.

Striking as is the disparity between the initial nucleoplasmic ratio and the ratio at the height of the lampbrush figure, the increase in surface area of the chromosomes during this period is even more striking. It is evident from a comparative study of nucleoplasmic ratios and of surface area of chromosomes that, while cytoplasmic volume increases at a proportionately much

greater rate than does nuclear volume, it actually lags behind the proportionate rate of increase of surface area of the most important structural element of the nucleus—the chromosomes. It must be borne in mind, however, that the chromosomes later undergo a regression preparatory to the meiotic divisions, while the egg continues to grow. What these facts actually mean is perhaps best deferred until the discussion of the function of the lampbrush chromosomes.

It should be admitted that the assumptions basic to these volumetric calculations are not entirely valid. The nucleus may be almost a perfect sphere in the earliest stages but it becomes somewhat eliptical and irregular in outline while the chromosomes are still in the pseudopodial stage. Thus calculations of nuclear volume must be approximations, but they are probably close approximations as the shape of the nucleus usually is not far from that of a sphere. The shape of the cell as a whole is variable, but it tends to be a sphere except for distortions owing to pressure of surrounding eggs. By averaging measured diameters of eggs cut in many different planes it was hoped that these variations would be smoothed out sufficiently to give a reasonably accurate average diameter for cells with any particular size of nucleus.

With these admissions in mind it is still clear that, however much the particular figures stated may be in doubt, there is a striking decrease in the nucleoplasmic ratio during the development of the lampbrush chromosomes.

## Chemical Considerations

Kodani's statement (1946) that methods for analysis of the chromosomes *in situ* "require great sensitivity and specificity because of the extraordinarily small quantities of the substances present in the chromosomes" applies with even greater force to the lampbrush chromosomes than to the salivary-gland chromosomes of which he wrote. Only two previous writers (Brachet and Painter) have attempted any chemical interpretation of the lampbrush chromosomes. Both noted the presence of thymonucleic acid in the main axis but attempted no further analysis.

This is not the place for a general review of chromosome chemistry, because very little indeed can as yet be applied on an experimental basis to the lampbrush chromosomes. But the pertinent data should be summarized. Known genetic data demand that there be a high degree of differentiation along the length of the chromosome. The proteins are the only known class of compounds which could offer this diversity. Yet only four proteins have been identified in chromosomes. Histone and protamine have been known for many years to enter into the composition of the chromosomes, especially those of fish sperm (Kossel, 1928). Caspersson (1936) using his ultraviolet absorption method, found these proteins, together with globulin, in the salivary-gland chromosomes. He believed that globulin runs the length of the chromosome, that protamine runs the length of the euchromatic parts, and that histone is confined to the chromatic bands. Because of this he thought it probable that histone forms the link between the nucleic acid and the protein skeleton of the chromosome. Mazia (1941), however, believes that histone forms the skeleton

of the chromosome, with protamine forming the link to the nucleic acid. He based his view on the removal of nucleic acid from the chromosomes by a preparation known to contain protaminase and on the readiness with which purified histone and nucleohistone form fibers, while protamine and nucleoprotamine fail to form fibers. He believes that the shrinkage of interband spaces by pepsin points to globulin as the "matrix" with which the interband spaces are swollen. Obviously the protein removed by pepsin can have nothing to do with the skeleton of the chromosome, as the chromosome remains intact following pepsin digestion. Nor can this protein form the link between the skeleton and nucleic acid, for the chromosomes remain Feulgen positive after pepsin digestion. Mazia has also found an incompletely characterized "nucleoprotein X" which will form fibers. Lastly, the Stedmans (1943) have reported an acid protein, chromosomin, which they believe to be the most important part of the chromosomes. Their work, however, has not been generally accepted.

Every investigator, with very few exceptions, has attested to the presence of thymonucleic acid as a major constituent of the chromosomes. Kossel (1928) suggested that this substance is bound by a salt bond to protamine or histone. Schultz (1941) has found evidence, using Unna's stain, that ribonucleic acid is also present in the chromatic bands of the salivary gland chromosomes, although most investigators have found this substance only in the nucleoli and the cytoplasm (Brachet, 1940*b*).

The chemical data of the present study are derived from enzyme experiments, from staining by the Feulgen method for thymonucleic acid, and from staining by Unna's method for ribonucleic acid. Attempts at staining by several color tests for proteins failed, probably because of the extremely fine dimensions of the fibers studied.

The results of nuclease digestion and staining by the Feulgen reaction show that thymonucleic acid is present in both the main axis and the pseudopodia early in stage I. The thymonucleic acid soon disappears from the pseudopodia, but it remains demonstrable in the main axis of the chromosomes throughout the three periods studied. Unna's stain, however, shows that ribonucleic acid is present in the chromosomes in demonstrable quantities only in stage III, when its presence is certain only in the loops; but some sections suggest that it may also be present in the main axis. Nucleoli are always positive for ribonucleic acid, as is the cytoplasm (except in the earliest part of stage I).

The results of digestion with proteolytic enzymes are less clear. The chromosomes are at all times undigestible by pepsin in the sense that their visible structure is not disrupted, nonetheless there is some evidence of a shrinkage such as Mazia and Jaeger (1939) have reported for pepsin-digested salivary gland chromosomes. In the smallest stage I cells, the pseudopodia appear a little less frequent and shorter than in control slides. Stage II and III chromosomes show no measurable difference from those of controls, but one gets the impression that the superior visibility of pepsin-digested chromosomes is in part caused by a slightly greater thickness of the threads. Taking these data together, it seems probable that pepsin removes a small amount of protein

from the matrix, thus resulting in a slight shrinkage of those materials remaining in the chromosomes. This shrinkage is sufficient that the very small pseudopodia of early stage I now blend into the main axis, while the larger pseudopodia simply shorten but remain individually visible.

Assuming that the proteins present in the lampbrush chromosomes are the same ones that have been identified in salivary gland and other chromosomes, then this pepsin-digestible protein must be either globulin or "nucleoprotein X" since neither histone nor protamine is digestible by pepsin. As the Feulgen reaction is undiminished following pepsin digestion, it seems unlikely that any significant amount of nucleoprotein has been removed. Thus the pepsin-digestible matrix protein of the lampbrush chromosomes may be tentatively identified as globulin.

Trypsin digestion proceeds in at least two stages distinguishable by the methods used in this study. The first protein attacked is one by which thymonucleic acid is attached to the skeleton of the chromosome. This is made visible by a diffuse Feulgen reaction throughout the nucleoplasm, which is completely negative in control slides. Second, the chromosome as a whole is destroyed. The loops are not destroyed any more rapidly than are the main axes. These proteins by which the thymonucleic acid is attached, and by which the structural integrity of the chromosome is maintained (skeletal proteins), were unaffected by pepsin digestion, hence they must be either histones or protamines. The fact that the thymonucleic acid is liberated after short periods of digestion while the chromosome as a whole is destroyed only after much longer digestion suggests that the two functions may be subserved by different proteins. As stated above, Mazia (1941) has found that purified histone and nucleohistone will form fibers, while protamine and nucleoprotamine will not. Further, he found that chromosomes treated with a solution known to contain protaminase become Feulgen negative. These facts suggest that the skeleton of the lampbrush chromosome is formed by histone, while the thymonucleic acid is attached to the skeleton by protamine.

The picture of the chemical structure of the lampbrush chromosomes which emerges from the present study, considered in relation to some previous studies on chromosome chemistry, is as follows: the structural basis of the chromosome is a histone chain, to which thymonucleic acid is attached through protamines. In the earliest stages this applies to pseudopodia as well as to the main axis. The thymonucleic acid soon disappears from the pseudopodia, to be replaced in stage III, and possibly in all stages, by ribonucleic acid. At all stages the chromosome is impregnated with a small amount of globulin "matrix." This is substantially the same picture which Mazia (1941) envisions for the salivary-gland chromosomes.

A word of caution regarding the interpretation of discussions of chromosome chemistry: terms such as histone, protamine, and globulin sound very definite, and cytologists sometimes forget that these are not definite substances. Rather they are classes of substances, and within any one of them there may be a great variety of specific compounds. Present microchemical methods have not permitted a localization of such specific variants in the

chromosomes, but it is quite possible that these several classes of proteins actually do furnish all of the variability which is demanded by the genetic analysis of chromosomes.

"Matrix" is similarly subject to misinterpretation. Its use often implies inert material, simply packing in the chromosome. As used in this paper, "matrix" refers to substances not concerned with the morphological integrity of the chromosome. No implication of inertness is intended. Indeed, it seems highly probable that the matrix proteins are highly active in the genetic system.

### The Function of the Lampbrush Chromosomes

In the absence of specific physiological experiments, discussions of the function of cellular structures must remain hypothetical. However some of the morphological and chemical results of this study do give a certain amount of insight into this problem. Few will doubt the essential correctness of Rückert's opinion (1892) that the lampbrush chromosomes represent a simulation of the resting structure, in order to permit intensive metabolism in these meiotic cells. As the greater part of the lampbrush substance does not enter into the formation of the chromosomes of the first meiotic division but is sloughed off, he reasoned that this material must be purely "somatic" in function, while only that chromatin entering into the formation of meiotic chromosomes is concerned with heredity.

This concept of two different functional types of chromatin, trophochromatin for somatic functions and idiochromatin for hereditary functions, was further developed long ago in a series of papers by Goldschmidt (1904*a*, 1904*b*, and 1910) and Goldschmidt and Popoff (1907). In these papers Goldschmidt pointed out that in most cells these two types of chromatin exists side by side, so that the cell may, in a sense, be said to be binuclear. This concept was originally suggested by the extrusion of chromidia from the nuclei of certain Protozoa. An actual separation of trophochromatin and idiochromatin into two distinct nuclei in each cell perhaps occurs only in the ciliates, with the micronucleus containing idiochromatin and the macronucleus containing trophochromatin. Although this work includes many errors in details, the main concept appears valid. As applied to the lampbrush chromosomes, this concept may be very fruitful and can now be related to specific chemical differences as well as to morphological entities.

In young stage I cells the chemical structure of the chromosomes is uniform throughout, at least on the level of analysis possible with present methods. Soon the thymonucleic acid disappears from the pseudopodia, to be replaced in stage III by detectable amounts of ribonucleic acid. Meanwhile, however, many nucleoli have arisen in contact with the loops, and these are all strongly positive for ribonucleic acid. The cytoplasm has also become positive, and deposition of yolk begins in the most strongly positive region, that is the periphery in *Amphiuma* or the perinuclear region in *Squalus*. It seems probable, then, that the lampbrush loops function as sites of transformation of thymonucleic acid into ribonucleic acid. This ribonucleic acid, together with an even greater amount of protein, forms the nucleoli. During stage II these reactions

apparently proceed too rapidly for either of the nucleic acids to accumulate in detectable quantities in the loops.

Somehow this nucleolar substance is transferred to the cytoplasm. The present study gives no clue to the mechanism of transfer. Duryee (1941), however, claimed to have observed such a transfer in living cells. He says that as a nucleolus comes into contact with the nuclear membrane, the two membranes fuse, then the double layer so formed breaks out, discharging the contents of the nucleolus into the cytoplasm and leaving the greater part of the nucleolar membrane as a new addition to the nuclear membrane. He believed that this is a regular method of growth for the nuclear membrane. While the present study does not support Duryee in this respect, neither does it contradict him. Schreiner (1918) observed connecting strands between nucleoli and mitochondria in epidermal gland cells of *Myxine*. She believed that nucleolar material trickled (aussickern) through the nuclear membrane.

Once in the cytoplasm, the nucleolar material undoubtedly is associated with synthetic processes, for the appearance of large quantities of ribonucleic acid in the cytoplasm marks the beginning of rapid growth of the egg, and deposition of yolk first begins in the region of greatest concentration of ribonucleic acid. A priori, it seems improbable that the rather simple ribonucleic acid is the only nucleolar material which is concerned with these synthetic processes in the cytoplasm. The far more complex proteins must be more than just carriers for the ribonucleic acid. Modern genetic theory conceives of the chromosomes as functioning through the formation of developmental enzymes, or other types of material which may influence the course of development (and cell reactions of adults). The proteins of these nucleoli, produced by the lampbrush loops and secreted in large quantity into highly active cytoplasm, may well be such developmental enzymes in visible form.

In terms of a modernized binuclearity theory of Goldschmidt, then, the main axis of the chromosome corresponds to the idiochromatin, while the loops, in which ribonucleic acid and proteins for use in the cytoplasm are synthesized, correspond to the trophochromatin, as do the same products in the nucleoli and in the cytoplasm. The lampbrush structure is primarily a mechanism for obtaining a surface area commensurate with the intense metabolism of the developing egg. But in addition to this it constitutes a partial separation between the idiochromatin which here appears to be associated with thymonucleic acid and the trophochromatin which here appears to be associated with ribonucleic acid.

## SUMMARY

In the development of telolecithal primary oöcytes of vertebrates, the chromosomes develop many side loops, because of which they have been called lampbrush chromosomes. A morphological and biochemical study of the development of these chromosomes has been presented.

These processes first appear as pseudopodia extending from the chromomeres. Soon the centers appear to break out, leaving loops, much as though soap films on wire loops had broken. These loops rapidly grow to become very long and slender, and during this growth they lose almost completely their

affinity for basic stains. Nucleoli in large numbers are formed in contact with the lampbrush loops. The loops as well as the main axis of the chromosome appear to have a chromomeric structure. The chromomeres of the main axis have been measured and have been found to be very near the limits of visibility. It has been pointed out that there is a close morphological correspondence between these chromosomes and the lampbrush chromosomes which Kodani (1941) has produced from salivary gland chromosomes of *Drosophila*.

Some conclusions regarding the chemical nature of lampbrush chromosomes have been drawn from experiments with Feulgen and Unna stains and with the enzymes nuclease, pepsin, and trypsin. The structural skeleton of the chromosome appears to be histone, while the nucleic acids are attached to this skeleton through protamine. In the main axis the nucleic acid is of the thymonucleic type, while in the loops this is rapidly converted to the ribonucleic type. The whole chromosome includes a small amount of "matrix" of globulin.

Functionally, the lampbrush chromosomes are regarded as agents for the synthesis of ribonucleic acid and enzymes, or other type of essential developmental agent, for use in the cytoplasm. The lampbrush form provides a surface area sufficient to permit the intensive metabolism of the growing egg. In these chromosomes there is a functional separation of idiochromatin (the main axis) and trophochromatin (the side loops).

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# FLAGELLATES OF THE CAECUM OF GROUND SQUIRRELS

BY
HAROLD KIRBY AND BRONISLAW HONIGBERG

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## INTRODUCTION

THE INTESTINAL protozoa of ground squirrels of the genus *Citellus* remained unknown until the appearance in 1926 of Becker's publications on the amoebae and flagellates of *C. tridecemlineatus*. Soon thereafter Sassuchin (1931) made a study of the protozoan fauna of the intestine of *Citellus pygmaeus* in Russia. It is a matter of considerable interest that, in spite of the wide geographical separation of the hosts, the flagellates and amoebae of the ground squirrels of Russia and Iowa were assigned to the same species. There are many other species of *Citellus*, and a knowledge of how much the protozoan fauna agrees in the different ones is desirable. Basic to that knowledge is a more complete and accurate description of the flagellates than can be found in the earlier publications. In furtherance of this subject, we have undertaken to study the flagellates in the caecum of the type host and of three species of *Citellus* in California.

An opportunity of studying the intestinal flagellates of *Citellus beecheyi beecheyi* was first given the senior author at the Hastings Natural History Reservation, Monterey County, California. Through the kindness of Dr. J. M. Linsdale, facilities were provided for collecting the protozoa from numerous ground squirrels and for making some studies at the place of collection.

Specimens of *Citellus lateralis chrysodeirus* were obtained in Yosemite National Park, with the permission of the Superintendent of the Park and the aid of the Park Naturalist. Later a number of the squirrels were trapped at Reds Meadow, Madera County, California. A collection of caecal contents from Mantled Ground Squirrels was made for us at Big Bear Lake, San Bernardino County, California. No specimens of those animals have been available for determination, but the distribution indicates that they belonged to the subspecies *Citellus lateralis bernardinus*. (See Howell, 1938.) For aid in arrangements necessary to secure the material from Big Bear Lake acknowledgment is due to Richard P. Maynard and Dr. K. F. Meyer. The caecal contents in potassium dichromate were used primarily for the study of *Eimeria*, but some information regarding the flagellates was secured.

Specimens of *Citellus beldingi beldingi* were taken at Tuolumne Meadows and at Reds Meadow. Four living *Citellus tridecemlineatus tridecemlineatus* were sent from Ann Arbor, Michigan, by Dr. William Burt. We make grateful acknowledgment to him for this essential material.

Studies were made of living flagellates when possible, sometimes with the aid of dark-field illumination. Film preparations from the caecum were fixed in Schaudinn's fluid or Hollande's fluid. Heidenhain's iron-haematoxylin was used as a standard stain, but a great aid in the study of some of the flagellates

was silver impregnation by use of activated protargol, usually after fixation in Hollande's fluid.

All the drawings that illustrate this paper were made by the junior author. For aid in providing for the work acknowledgment is made to the Research Committee of the University of California.

## **Chilomastix magna** Becker

(Plate 30, *a–o*)

*Chilomastix magna* was found present in abundance in the caecum of all four specimens of *Citellus tridecemlineatus* from Michigan. Becker (1926) reported it in all of twenty specimens of this squirrel. In *Citellus beecheyi* at Hastings Natural History Reservation and in *Citellus beldingi* at Tuolumne Meadows and Reds Meadow it was less frequent, being found in small numbers in some squirrels and not at all in others. Sassuchin (1931) reported the flagellate in 60.7 per cent of 242 *Citellus pygmaeus* in Russia. In *Citellus lateralis* we did not observe this flagellate.

*Chilomastix magna* has a size large in comparison with that of *C. mesnili*, but flagellates of the genus from many other hosts are as large or larger. Fixed specimens from *Citellus tridecemlineatus* ranged in length from about 12.5$\mu$ to 22$\mu$ and in width from 8$\mu$ to 10.5$\mu$, fifty-two specimens having an arithmetic mean in these dimensions of 15$\mu \times$ 8.5$\mu$. The flagellates from *Citellus beecheyi* had a length of 13.5$\mu$ to 23$\mu$, a width of 7.5$\mu$ to 11.5$\mu$, and a mean of 16.5$\mu \times$ 8.5$\mu$. These measurements are in essential agreement with those reported by Becker (1926), although he found the range to extend about 2$\mu$ lower in length and width. Flagellates of the genus *Chilomastix* in many other rodents are of comparable size or a little smaller; but *C. aulastomi*, *C. caulleryi*, and *Chilomastix* sp. from *Bufo vulgaris* described by Bishop (1935) are larger.

The shape of the body is that characteristic of the genus; the anterior end is broadly rounded and posteriorly there is a moderately long, pointed caudal process (pl. 30, *a*, *b*). The length of the caudal process, which is included in the body length given above, ranged from 3.5$\mu$ to about 6$\mu$ in specimens from *C. tridecemlineatus* and from 3.5$\mu$ to about 7$\mu$ in specimens from *C. beecheyi*, averaging 4 6$\mu$ in each.

Studies of the flagella were made by dark-field illumination in living material from *Citellus tridecemlineatus*. There are three anteriorly directed flagella about 9–10$\mu$ long (pl. 30, *c*). They are the same thickness for all of their length or appear somewhat enlarged at the distal end. In dark field the terminal part has a more intense brightness. The posteriorly directed flagellum is considerably shorter than the others and appears to be less stout, but still it shows very plainly in dark field. Its undulatory activity is rapid and there is a rather wide translation in its position, but it does not leave a particular region over the cytostome. When the flagellar movements slow down after the flagellate has been for some time in a paraffin-sealed preparation, the activity of the anterior flagella is reduced more than that of the cytostomal flagellum, which may continue rapid movement when the other flagella move very slowly.

It may be observed both in living and prepared specimens that the three anterior flagella originate separately from the body and are distinct from one another for all their length (pl. 30, *a, b, c*). There is no common grouping at the base as in trichomonads. There are three granules at the anterior end of the body, one at the base of each of the flagella. Two of the granules are close together and the third is separated from them by a somewhat greater distance (pl. 30, *c*). The third granule is closer to the anterior edge of the cytostome than the others (pl. 30, *b*). The third flagellum is often directed forward parallel to the others; but at other times it is directed away from them toward the left, sometimes almost parallel to the transverse axis of the body (pl. 30, *a*), or even directed somewhat posteriorly.

The fourth flagellum was best seen in preparations that had been fixed in Champy's fluid and stained in Heidenhain's iron-haematoxylin. As it appears in stained material, it originates under the anterior margin of the cytostome and passes posteriorly above the cavity of the cytostome. In many specimens it turns outward, free of the opening (pl. 30, *a*). At its proximal end this flagellum evidently runs from within the anterior depression through the cytoplasm to meet a granule or granule-complex anterior to the nucleus and close to its membrane. For certainty in demonstration of this granule, the origin of the fourth flagellum, and possible fibrils that connect the granule to the three granules of the anterior flagella, the material studied did not suffice. There are indications of the existence of interconnecting fibrils, but errors resulting from preconceptions are easily made in connection with supposed structures of that sort.

Beginning at the anterior end of the body at a distance of 2$\mu$ or 3$\mu$ ventral to the apex, and extending along the region ventral to the nucleus, is the cytostome and the complex differentiations associated with it. It has not been possible for us to obtain a complete understanding of this structure, but a number of things about it have been clearly observed.

The depression passes deeply into the cytoplasm; it is not superficial. Its anterior edge has a rounded margin and at this margin is a fibril which there stains deeply with iron-haematoxylin and is fairly thick (pl. 30, *d*). The fibril passes posteriorly in the right and left margins of the cytostome. On the left side it is confined to the superficial border and is rather slender. After following a fairly straight course, it comes to an end (pl. 30, *b, f*). On the right border the fibril not only extends along the whole length of the cytostome, but posteriorly it turns in a loop and passes deeply into the cytoplasm. About midway between the anterior edge of the cytostome and the loop there is a curvature of this structure toward the left, a feature which gives the cytostomal area the appearance of being constricted in the middle (pl. 30, *b*). Posterior to the curvature the structure stains deeply and is not a simple fibril but a band broadened in a direction vertical to the surface. Its band form can be seen readily when the cytostome is observed at the left or right margin of the body. The posterior part of this filament shows clearly in some specimens and is not visible in others that are similarly stained, so there seems to be variation in its degree of development.

The posterior part of the looped filament, which lies deep in the cytoplasm, runs parallel to a cytoplasmic differentiation of elongate, somewhat rectangular form which begins anteriorly in the region of the anterior border of the cytostome (pl. 30, *f*). When this differentiated region is observed in a position where the cytostome faces the observer, it bends under the anterior part of the cytostome to the region of a granule anterior to the nucleus to which the flagella may ultimately connect. Observed from this aspect the structure is not very wide. Seen from a position at right angles to this, with the cytostome at the right or left edge of the body, it appears very broad. It is not an empty tube or sac, but is a cytoplasmic structure that appears vacuolated in this fixed material. Its composition resembles that of the cytoplasm elsewhere, but it appears denser. In its posterior part it often continues without any sharp demarcation into the general cytoplasm. It cannot be regarded as a definite mastigont structure, but seems rather to be a differentiated region of cytoplasm associated with the pocket into which the cytostome leads.

There is reason to believe that the open cytostome does not occupy the whole region from the anterior margin to the posterior loop. The posterior part of the right fibril, both at the loop and for a distance anterior to it, appears not entirely superficial in position. The cytostome may actually comprise only the oval region anterior to the curvature, between the left fibril or margin and the less deep-staining part of the right fibril.

The nucleus (pl. 30, *g–o*) is spherical and ranges in diameter from 3$\mu$ to 4$\mu$ averaging about 3.5$\mu$. In the nucleus, granules are dispersed in the interior or, exceptionally, aggregated in a central chromatic mass. There is peripheral chromatic material which is often massed in one or two plaques against the nuclear membrane. Frequently a plaque lies against the anterior edge of the nucleus. The plaque or plaques vary in size, form, and position. Sometimes there are no plaques, but the peripheral chromatic material is distributed in smaller bodies interior to the nuclear membrane. The peripheral chromatic material stains deeply with iron-haematoxylin and impregnates with protein silver. It also stains with Delafield's haematoxylin.

It is probable that the various species assigned to *Chilomastix* constitute a genus which is homogeneous in basic structure, and that the discrepancies in existing accounts will eventually be modified and reveal this homogeneity.

*Chilomastix magna* seems from Becker's account to differ from all other species in that "there is often a tuft of short bristly cuticular projections in the region of the flagella." These projections have not been seen in the material we have studied, and it is likely that there are no such structures belonging to the flagellate. Microörganisms lying near the base of the flagella might result in that appearance.

The existence of three separate granules from each of which an anterior flagellum originates, is in agreement with descriptions of some other species (Dobell and O'Connor, 1921; Bělař, 1921; and Bishop, 1935). In *Chilomastix magna* these granules appear constant in position at the anterior edge of the body and do not form a compact mass. The number of anterior flagella seems to be constantly three.

In the present studies of *Chilomastix magna* no evidence has been found that the three anterior flagellar granules are connected directly to one another or that any one of them is directly related to the cytostomal flagellum or to the pericytostomal fibrillar organization. The observations on the ground squirrel flagellate in this respect do not support the accounts of *Chilomastix* by Kofoid and Swezy (1920), Becker (1926), or Grassé (1926). The cytostomal flagellum has a separate origin which probably is like that shown by Bishop (1935) in her account of *Chilomastix* from *Bufo*. In that species there is a fourth granule posterior to the others. In *C. magna* there is a stainable body close to the nucleus, as Chang (1935*b*) found in *C. oblonga;* but whether it may consist of two or three blepharoplasts, as Chang supposed, was not determinable.

Morénas (1938) affirmed the existence of an undulating membrane in *C. bettencourti,* as Kofoid and Swezy (1920) and others had done in other species. Morénas based his statement on his observations in living material of regular undulatory movements of the cytostomal flagellum. Our dark-field observations on undulatory movements in one fixed region of this flagellum in *C. magna* gave an impression of an undulating membrane, but it should not be supposed that one can therefore affirm the existence of that structure. Certainly that is not the only possible interpretation of the observations. The cytostomal flagellum passes over a deep depression, and there is nothing to show where such a membrane could be attached. The flagellum is certainly largely free in many fixed specimens.

In most accounts of *Chilomastix,* the cytostome has been considered to be extended as far posteriorly as the loop of the fibril, and to be broadly rounded at both ends sometimes with a narrowed isthmus in the middle part. There is an exception to this concept in Chang's account of *Chilomastix oblonga.* (1935*b*). He observed the cytostome to be pointed posteriorly, and not to be extended in that region to the looped fibril. There is evidence that a similar situation may obtain in *Chilomastix magna,* though in it the cytostome may not extend so far posteriorly and has more of an oval shape. The pouch interior to the cytostome is extended more widely, so that there is a shelf of protoplasm overhanging the pouch in the posterior part.

The structural differentiations associated with the cytostome and the pouch into which it leads have been described and interpreted in diverse ways. Prior to the work of Kofoid and Swezy (1920) authors generally spoke only of siderophile margins of the cytostome. Chalmers and Pekkola (1918) had, however, supposed that the siderophile rim, continuing around the cytostome posteriorly, connects anteriorly on each side to a separate blepharoplast. They wrote, somewhat vaguely, of a homology of the "outer siderophilous rim" to the parabasal or chromatic thickening at the base of the undulating membrane of *Trichomonas,* and believed that it grows backwards from the blepharoplast.

Kofoid and Swezy (1920) were influenced by Chalmers and Pekkola's account in considering that the anterior connections of the siderophile rim are separate, each side to a blepharoplast, but they also observed that the

structures are not continuous posteriorly; thus according to their concept there are two separate fibrils, one on each side of the cytostome, connected separately to blepharoplasts. These fibrils, they wrote, do not actually lie in the rim of the cytostome, which is completely encircled by an inner peristomal fibril not directly related to either of them.

Many later accounts have been influenced by the precise description and diagrams published by Kofoid and Swezy (Leiva, 1921, *Chilomastix intestinalis;* Boeck and Tanabe, 1926, *Chilomastix gallinarum;* Becker, 1926, *Chilomastix magna;* Grassé, 1926, *Chilomastix caulleryi*) ; but our observations on the cytostomal structures of *C. magna* are more nearly in agreement with the description by Bělař (1921) of *Chilomastix aulostomi* and that by Bishop (1935) of *Chilomastix* sp. from *Bufo vulgaris* than with Becker's account of the ground squirrel flagellate. Bělař remarked that the supporting fibrils (Stützfibrille and Parastyl) are connected neither to one another nor to a basal granule, but end anteriorly, as well as posteriorly, free in the plasma; the Parastyl bends around the anterior margin of the cytostome. Bishop (1935) noted that in the full grown flagellate the peristomial fibrils are not connected with the basal granules of the flagella. Geiman (1935) remarked that he saw evidence in cysts of *Chilomastix* from *Macacus irus* that the cytostomal fibril is only one structure, so that the two fibrils are connected anteriorly and do not run separately to blepharoplasts. He found that the anterior curve is in proximity to the blepharoplast from which the buccal flagellum extends. In the division process, however, in the species he studied, new buccal fibrils are reported to originate in connection with blepharoplasts.

Becker's diagram (1926, pl. 1, fig. 12) shows the peristomal fibrils of *Chilomastix magna* ending anteriorly each in a basal granule, and the two granules are connected by a transverse strand. This diagram would be brought into agreement with our observations if the granules and strand are actually only part of the fibril on the left side of the cytostome which encircles the anterior margin; an impression of granules could be given in focusing when no such structures actually exist.

The long posterior extension of the right fibril, following the loop, was not seen by Becker in this species, but it is shown by Sassuchin (1931) in *Chilomastix magna* from *Citellus pygmaeus*. A similar posterior prolongation of the fibril is shown by Geiman (1935, pl. 3, fig. 31) in *Chilomastix intestinalis*.

Certain published drawings of *Chilomastix* show that the posterior part of the right cytostomal fibril, leading to and including the loop, is not a simple structure. It appears bordered by a differentiated area, as is shown in plate 30, *b*. Bishop (1935) stated that this gives the impression of a second and deeper fibril in *Chilomastix* from *Bufo*. Geiman (1935) showed the broadened, often darker area along the right fibril in *Chilomastix mesnili* from man and monkeys and in *C. intestinalis*. He wrote of peristomal fibrils appearing as faintly staining shadow fibrils under the buccal fibrils, and represented the structure in many figures of trophics, cysts, and division stages. In 1910 Alexeieff had shown a similar differentiation along the right fibril of *Chilomastix caulleryi*, and it is indicated in the figures by Boeck and Tanabe (1926)

of *Chilomastix gallinarum*. Observations made on *Chilomastix magna* suggest that the object seen is a broadened structure rather than a simple fibril. It narrows to a fibril again in the posterior part of the loop and the posterior continuation.

The differentiated cytoplasmic structure associated with the cytostomal depression was called by Becker a parabasal body, but that term is not suitable for it. It is not so well-defined and constant a structure as the parabasal body, and it is not always sharply delimited, at least in its posterior part. Its staining reactions are not those of a trichomonad parabasal body, nor is its composition in any way similar. Crouch (1936) reported that "Becker's parabasal body" is present in *Chilomastix instabilis* from a woodchuck. Geiman (1935) found a similar differentiation in *Chilomastix intestinalis*, and stated that he had seen it in isolated cases in all species of the genus studied. In the guinea pig *Chilomastix* he found it both in some adults and in division stages, where it is related anteriorly to the blepharoplasts, along with the buccal fibrils, and posteriorly fades out into the general cytoplasm with no sharp demarcation.

Grassé (1926) described as a parabasal body in *C. caulleryi* a structure of rodlike form close to the nuclear membrane, attached to a blepharoplast in one instance directly (where it lies on the anterior surface of the nucleus, his pl. 19, fig. 314), and in another instance by a relatively long filament (where it lies more posteriorly against the nuclear membrane, his pl. 19, fig. 313). Structures that look like this appear in relation to the nucleus of *C. magna*, but they are plaques of peripheral chromatin within the nucleus. Grassé's drawings do not make it clear that he may not have represented a structure of that sort, having been mistaken in regarding it as exterior to the nucleus. In the exhaustive monograph of Duboscq and Grassé (1933) on the parabasal apparatus of flagellates there is no reference to the parabasal body described by Grassé in *Chilomastix caulleryi*.

The detailed account by Dashu Nie (1948) of *Chilomastix intestinalis* appeared while this article was in press. There is a similarity between *C. magna* and that species in the size and shape of the body, the relative size and shape of the pouchlike cytostome, the length and position of the anterior flagella, and the position and structure of the nucleus. The body length, as he records it, is a little greater, though the width is a little less than in *C. magna*. According to his description, the border of the cytostome is supported by two fibrils, but these are shown to be joined to one another around the anterior margin, so the appearance is that of a continuous fibril as in *C. magna*. The left cytostomal lamella was not included in our analysis of the structure of *C. magna;* the characteristics of that structure in the ground squirrel flagellate must be determined by further study. Dashu Nie did not observe a free flagellum either in or protruding from the cytostome. Instead, he found an undulating membrane following a course close along the right cytostomal fibril. The short, posteriorly directed flagellum is plainly evident in *C. magna;* whether or not there may be a membranous attachment is a matter of uncertainty, but in both living and fixed material there is often a separate, free flagellum.

It is not easy to differentiate on morphological grounds the several species

of *Chilomastix* that have been described from rodents. Certain differences have been indicated in size, shape, nuclear position, and nuclear structure, but these are not easily evaluated. Careful study is necessary to establish their significance.

### Taxonomic Summary*

#### Chilomastix magna Becker

*Chilomastix magna* Becker, 1926, Biol. Bull., 51: 288, pl. 1, figs. 1, 2.

*Type host.*—*Citellus tridecemlincatus tridecemlineatus* (Mitchill). Iowa. (Becker, 1926.) Michigan. Museum Vert. Zoöl., Univ. Calif. 96797 ♀ Xenosyntype slides TP-690: 15, 18, 24, 27.

*Additional hosts*—

*Citellus pygmaeus* Pallas. Kazakstan, R. S. F. Soviet Republic. (Sassuchin, 1931.)

*Citellus beecheyi beecheyi* (Richardson). Hastings Nat. Hist. Res., Monterey Co., Calif. Homoeosyntype slides GP-681: 4, 15, 16, 19.

*Citellus beldingi beldingi* (Merriam). Tuolumne Meadows, Yosemite Nat. Park, Calif. Homoeosyntype slides GP-1075: 3, 12.

*Diagnosis.*—(From type host): length 15 (12.5–22)$\mu$; width 8.5 (8–10.5)$\mu$; caudal process, included in above length, 3.5–6$\mu$ long; three anteriorly directed flagella, originating separately at base, 9–10$\mu$ long; recurrent flagellum about half this length, not attached in membrane; cytostomal depression deep, bordered on left and right margins by a fibril that continues around the anterior edge, two fibrils separately joined to blepharoplasts not present; fibril on right margin turned in a loop posteriorly and passing deeply into cytoplasm; elongated cytoplasmic differentiation present near the inner region of the cytostome; no parabasal body present; nucleus anterior, spherical, diameter 3–4$\mu$.

## Monocercomonoides pilleata n. sp.

(Pl. 31, *a–h*)

Becker (1926) reported from *Citellus tridecemlincatus* a small flagellate which was usually present in large numbers in the caecum, and which he designated *Trichomonas* sp. Studies of that flagellate have convinced us that it is a species of *Monocercomonoides*. It occurs in all ground squirrels of the species *C. beecheyi, C. beldingi,* and *C. tridecemlincatus* that we have examined, and in them it is by far the commonest and most abundant flagellate. It was found in only one of nine individuals of *Citellus lateralis chrysodeirus* taken near Rainbow Falls, and in the caecum of that individual host it was the only flagellate present. In *Citellus pygmaeus,* Sassuchin (1931) did not list this flagellate separately, but his figures 48 and 49 indicate that he probably included it with the species he named *Tetratrichomastix citelli.*

* The terms used in designation of type slides have the following meaning:
SYNTYPE: Slides bearing specimens that come from the type host and upon which the original description is based.
XENOSYNTYPE: Slides from the type host selected for representative specimens, but not bearing the specimens of the original type designation or original description.
HOMOEOSYNTYPE: Slides bearing specimens from other hosts than the type host, the specimens having been compared with the original syntypes or xenosyntypes and found to be conspecific.
HYPOSYNTYPE: Slides bearing specimens used in correcting or extending the account of the species, but not from the type host and not compared with the original types.

Becker stated that "*Trichomonas* sp." is elliptical in form and 6.5–10$\mu$ in length, with an axostyle projection of 2–3$\mu$. Fifty specimens in our material from *Citellus tridecemlineatus* ranged in length from 5.7–8.0$\mu$, in width from 3.4–5.7$\mu$, averaging 6.9 × 4.6$\mu$. Flagellates from *Citellus beecheyi* ranged in one series from 5.2–9.1$\mu$ × 3.4–8 0$\mu$, and, in another from 5.7–10.9$\mu$ × 4.0–6.9$\mu$, averaging in the two series 6.9$\mu$ × 4.6$\mu$ and 8.6$\mu$ × 5.7$\mu$. The projecting axostyle has a length of about 1–4$\mu$, averaging about 2$\mu$.

Studies were made of living flagellates from *Citellus beecheyi* observed by dark-field illumination. These showed a body form rounded anteriorly and posteriorly, with a projection of the axostyle of about 2$\mu$. The length in this living material ranged from about 7$\mu$ to 11.5$\mu$. Observations on the flagella and the activity of *Monocercomonoides citelli* were made on flagellates from both *C. beecheyi* and *C. tridecemlineatus*.

There are regularly four flagella, although a few specimens were seen in which one was missing. Once, a flagellum was seen to become detached while the specimen was being observed in dark field. One of the flagella is constantly recurrent and is applied to the body surface in the anterior part of its length. This flagellum is longer than the others; in living material it ranged from 10–20$\mu$, and in silver preparations 12–21$\mu$, averaging 15.5$\mu$. The other three flagella ranged from 7–11.5$\mu$ in living material and in silver preparations from 8–13$\mu$, averaging 9.75$\mu$.

The three anterior flagella are entirely separate from one another to their base, never forming a group as in *Trichomonas* (pl. 31, *a*). They extend in different directions from the place of leaving the body. In what appears to be normal activity they are often directed backward over the surface of the body. Some living flagellates were observed in which these flagella were not at any time flung forward beyond a plane transverse to the apical end of the body; in others they were at times directed anteriorly, and in preparations they may be found fixed in both positions. The flagella move with great rapidity when the flagellate is in good condition. When kept in one field under dark-field illumination, the flagellate is rapidly affected, the body rounding up and the flagella soon stopping activity, the recurrent flagellum often becoming motionless before the anterior flagella do. The movements and interrelations of the flagella are very different from those of trichomonads.

It may be clearly seen in protargol preparations that each of the flagella has a terminal filament which is often 2–3$\mu$ long (pl. 31, *f*). This is different from the situation in trichomonads, in which only the posterior flagellum regularly ends in a filament, the anterior flagella terminating in knobs or deeply impregnating granules.

The organelles connect to two blepharoplasts at the anterior end of the body; the blepharoplasts are separated from one another by about as much distance as the diameter of nucleus (pl. 31, *a, d, f*). To one of the blepharoplasts two anterior flagella connect; the other gives origin to the third anterior flagellum, the longer recurrent flagellum, and the axostyle (pl. 31, *f*). The two blepharoplasts are joined to one another by a transverse filament (pl. 31, *a*).

The recurrent flagellum is applied to the surface of the body for about half

the body length, or somewhat more (pl. 31, *f*). At the periphery of the body, along this region of adherence of the flagellum, there is a differentiated structure that is plainly and regularly demonstrated in protargol preparations (pl. 31, *b, c, e, f*), but is not evident in iron-haematoxylin stains. In the silver preparations it is so regularly shown, and has so definite a structure that we feel certain that it is truly an organelle of the flagellate, and is not a result of fortuitous silver deposition. The structure suggests the costa of a trichomonad in its morphology and relationship to the trailing flagellum, but it differs from that in its failure to stain by iron-haematoxylin. It originates at that one of the blepharoplasts which gives origin also to the recurrent flagellum and the axostyle. It is a rod of fairly uniform size for all of its length, which reaches to or somewhat beyond the middle of the body. The flagellum typically parallels the rod for all of that length, but sometimes it is separated from it in the posterior part, and occasionally entirely.

The axostyle is a rod which in specimens of larger size often has a diameter of about 0.5–0.75$\mu$. It is not enlarged in its anterior part, which passes in close contact with one side of the nucleus and ends in the region of one of the blepharoplasts (pl. 31, *a, c, e*). Many specimens show a definite connection to that blepharoplast. In iron-haematoxylin preparations it is often well stained. There is much variation in the degree of impregnation of the axostyle by silver. In some specimens it is solidly black in all its part posterior to the nucleus, and in others it is light. Moderately impregnated specimens show a stain of the sheath of the axostyle, and of granules or patches of stained material that either lie against or are incorporated into the sheath.

The posterior end of the axostyle normally projects for a short distance from the posterior end of the cytosome (pl. 31, *a, b, f, h*). The projecting part is sharpened like a pencil point, and either is not greater in diameter than the preceding trunk, or is somewhat enlarged in spearhead form. Often there is in fixed material a greater amount of projection, including part of the trunk as well as the sharpened end; but it is not likely that this is representative of conditions in the normal living flagellate. At the beginning of the region of sharpening, there is an iron-haematoxylin staining ring which is closely applied to the axostyle. The position of this ring is constant in relation to the axostyle; it may coincide with the posterior end of the cytosome, as is probably the normal condition, or it may be carried to a distance from the body with a greater amount of axostylar projection.

A structure that in position and staining reactions resembles the pelta of *Pentatrichomonas hominis* is present in the anterior part of the body (pl. 31, *h*). It may be demonstrated very clearly by silver impregnation. The pelta is a membrane that passes transversely around the nucleus (pl. 31, *b, c, e*). It is broad at its beginning, which is in close proximity to that one of the blepharoplasts to which the recurrent flagellum and axostyle connect. It curves transversely, and in its distal part it is tapered, ending as a sharpened membrane that is commonly prolonged in a filament several microns long. In overimpregnated material the pelta appears as a continuous cap. When the details of its structure can be seen, it appears to be very consistent in shape.

When observed from a partly anterior aspect, it sometimes shows a crescentic form (pl. 31, *g*).

The pelta is not completely demonstrated in iron-haematoxylin preparations, and the staining of the nucleus then makes it difficult to study. Its presence would not have been recognized without the silver preparations. Nevertheless, parts of the pelta may be stained by iron-haematoxylin. Often the anterior, curved edge is stained, and this may appear to be a heavily stained curved bar extending between the blepharoplasts. Parts of the membranous structure elsewhere, especially at an optical section of a curve of the membrane, can sometimes also be made out.

No parabasal body has been seen in *Monocercomonoides pilleata*. The studies that have been made of well-impregnated silver preparations constitute a reasonably sound basis for concluding that probably there is no parabasal body.

The nucleus is situated always in the anterior part of the body with its anterior margin close to the blepharoplasts (pl. 31, *a*). It is spherical with an average diameter, in fixed material, of 2.3$\mu$, and a range of 1.7–3.4$\mu$. In material that has been much destained after iron-haematoxylin, a large spherical endosome may remain deeply stained while in the region of the nucleus around it there is little or no chromatic material. The endosome is generally central in position, but sometimes it is shifted toward one side of the nucleus. In heavily stained preparations the contents of the nucleus may be stained black throughout. According to the degree of destaining, the zone surrounding the endosome may be seen to be occupied by a varying amount of material; sometimes it is present throughout the zone, or it may be largely restricted to the region adjacent to the nuclear membrane.

Clear areas are present in the cytoplasm adjacent to the nucleus in many specimens of *Monocercomonoides pilleata*. The commonest position for a clear area is adjacent to the side of the nucleus opposite the axostyle. There are sometimes also clear areas adjacent to the posterior part of the nucleus, or adjacent to the region where the axostyle extends along the nucleus. The extent and boundaries of these areas vary greatly; often no clear areas are present. The clear area against the nucleus opposite to the axostyle may be especially well defined and crescentic in shape in optical section. There is no sharp boundary of these areas that has the character of a membrane. The regions appear to be no more than part of the general cytoplasm from which granules and stainable material are absent. The boundary of the granular cytoplasm adjacent to the clear area may or may not appear as a relatively even edge.

Wenrich (1946) stated that the genus *Monocercomonoides* Travis, 1932, has species in scarab beetle larvae, crane fly larvae, mole crickets, cockroaches, wood-eating roaches, termites, amphibians, and mammals. Among mammals, he mentioned its occurrence in *Peromyscus, Marmota, Thomomys,* guinea pigs, and rabbits. Several species from mammals have been named. *Monocercomonoides hassalli* (Cunha and Muniz, 1927) was originally described as *Monocercomonas caviae* by Cunha and Muniz (1921). The species of *Monocercomonoides* in marmots is probably the flagellate that was described as

*Trichomonas digranula* by Crouch (1933). *Monocercomonoides cunhai* (F. da Fonseca, 1939*b*) occurs in abundance in the caecum of *Coelogenys pacca* in Brazil. *Monocercomonoides caprae* Das-Gupta, 1935, was found in the rumen of goats in India.

The species in mammals are similar to one another in size and shape. They all possess four flagella in two pairs. In *Trichomonas digranula* a fifth flagellum, recurrent and applied to the surface of the anterior part of the body, over a costa, was described. The systematic position and structural features of that marmot flagellate are uncertain, pending further information about it.

In most species the four flagella are more or less equal in length and are shown free of contact with the body surface Grassé (1926) mentioned that in *M. melolonthae* one of the flagella is sometimes much longer than the other three and trails posteriorly, as was also reported by Jollos (1911) in *M. cetoniae,* which Grassé considered to be a synonym of *M. melolonthae.* That arrangement of the flagella, which is a regular feature of *M. pilleata,* occurs also in *Monocercomonoides lacertae,* as described by Tanabe (1933).

Drawings that accompany the descriptions of *Monocercomonoides cunhai* (F. da Fonseca, 1939*b*) and *M. caprae* (Das-Gupta, 1935) show a relatively large, rounded protuberance anteriorly, between the two blepharoplasts. A similar protuberance is present in some species of *M. pilleata* (pl. 31, *h*), in which it is the region occupied by the pelta. No structure that corresponds to the pelta has been described in any other species of *Monocercomonoides,* but very probably it exists at least in some of the other mammalian forms. Possibly Das-Gupta's report of the chromatic line bordering the anterior edge of the body of *M. caprae* resulted from observation of an edge of the pelta.

Tanabe (1933) stated that in *M. lacertae* the cytostome is an opening at the anterior edge of the body, with a short cavity leading into the interior along the major axis of the body. Cunha and Muniz (1921) identified a clear crescentic area alongside the nucleus of *M. hassalli* as the cytostome, the existence of which they considered to be a characteristic feature of the species, as it had not been described before in flagellates of the genus. A similar clear area in *M. cunhai* is referred to as a cytostome by F. de Fonseca (1939*b*). Bütschli's statement (1884) about a depression near the base of the flagella as possibly a mouth opening—aside from uncertainty whether he was writing about the flagellates in the present genera *Monocercomonas* or *Monocercomonoides*—probably does not refer to this interior clear zone. A similar statement was made by Das-Gupta about a slight depression anterior to the nucleus of *M. caprae.*

Structural differentiations comparable to those mentioned by authors as the cytostome can be seen in many specimens of *M. pilleata.* But we have failed to find sound evidence, either by study of the literature or by observation, that comparison with a definitely formed cytostome, like that in ciliates, is in any way justified. The clear regions beside the nucleus are deep in the body, surrounded by other cytoplasm. Only the part where this region might open to the surface anteriorly could properly be termed a cytostome; but there is no evidence for the existence in *M. pilleata* of such an opening.

## Taxonomic Summary

**Monocercomonoides pilleata n. sp.**

*Trichomonas* sp.?, Becker, 1926, Biol. Bull., 51: 293, pl. 1, figs. 17, 19.

*Type host.*—*Citellus tridecemlineatus tridecemlineatus* (Mitchill). Michigan. Museum Vert. Zool., Univ. Calif. 96797 ♀. Syntype slides GP-690: 2, 3.

*Additional hosts*—

*Citellus beecheyi beecheyi* (Richardson). Berkeley, California. Homoeosyntype slides GP-693: 2, 3.

*Citellus beldingi beldingi* (Merriam). Tuolumne Meadows, California. Homoeosyntype slide GP-1074: 3.

*Citellus lateralis chrysodeirus* (Merriam). Yosemite Nat. Park, California. Homoeosyntype slide GP-1077: 3.

*Diagnosis.*—Length 5.7–8$\mu$ in type host, average 6.9$\mu$, range 5.2–10.9$\mu$ in *C. beecheyi;* width 3.4–5.7$\mu$ in type host, average 4.6$\mu$, range 3.4–8$\mu$ in *C. beecheyi;* four flagella in two pairs, three directed variously, length 7–13$\mu$, one recurrent and adherent to body surface for about half the body length, length 10–21$\mu$; rodlike, silver-impregnating structure at periphery, along region of adherence of flagellum; axostyle rodlike, slender, projecting posteriorly 1–4$\mu$, with ring at place of emergence; curved, pointed, silver-impregnating membrane, the pelta, in peripheral cytoplasm of anterior part of body; nucleus anterior, spheroidal, 1.7–3.4$\mu$ in diameter.

## Hexamitus pulcher Becker

(Pl. 32, *a–i;* pl. 33, *g, h*)

*Hexamitus pulcher* was found by Becker (1926) in most specimens of *Citellus tridecemlineatus* that he examined, and the flagellates were present in abundance in the caecum of the four ground squirrels of that species which we studied. Sassuchin (1931) reported *H. pulcher* in the majority of specimens of *Citellus pygmaeus.* We found it to be present commonly in *Citellus beecheyi.* In *Citellus beldingi* it was present in only two of the animals examined at Reds Meadow, and in none at Tuolumne Meadows; in the infected ones it occurred in small numbers in the caecum. The flagellate was not found in *Citellus lateralis chrysodeirus.* Most of our studies have been made on the flagellates from the type host.

Becker recorded the size as 8–10$\mu \times$ 6–7$\mu$, and Sassuchin reported it to be 6–8$\mu \times$ 5–6$\mu$. Fifty specimens from *C. tridecemlineatus* ranged in length from 6.3–10.3$\mu$ and in width from 3.4–6.3$\mu$, averaging 8.6$\mu \times$ 4.6$\mu$. These measurements cover the whole range in both the apparently separate groups in the accounts by Becker and Sassuchin. The normal shape of the species is more slender than that indicated in Becker's account.

Our studies have been made on flagellates some of which were stained by iron-haematoxylin, and others impregnated by protein silver. In specimens prepared by the former method, which has been used in practically all studies of the genus, little was observed at first that differed essentially from published accounts. Silver impregnation has not, so far as we know, been used before in studies of *Hexamitus,* and it has revealed some features of considerable interest. After studies had been made of the diagrammatically clear

silver preparations, it was often possible to find similar features, which we had not previously recognized, in the iron-haematoxylin stained material.

The six anterior flagella become free at positions around the sides of the body at a level several microns posterior to the anterior end (pl. 32, *a, d;* pl. 33, *h*). In accordance with the manner of their origin, the anterior flagella all tend to be normally directed backward. That position has been observed in dark-field studies of living flagellates, as well as in fixed material. The free parts of the flagella have a length of 5–8$\mu$.

The free parts of the flagella originate at the posterior ends of six rodlike, silver-impregnating differentiations, which are situated at the periphery of the body lateral to the nuclei (pl 32, *f*). The length of these rods is 1.7–3.4$\mu$. Four of them are in two pairs, in which they meet anteriorly to form V's (pl. 32, *e, f, g*). These two V's have been observed in all specimens studied. The rods forming the limbs of the V meet at the apex, but we are unable to state whether or not they are actually joined, as they appear to be. The remaining two rods are single. In most specimens these rods alternate with the V's (pl. 32, *c, f*), but in some they are paired (pl. 32, *g*).

It has not been possible for us to trace the flagella anteriorly beyond the point of meeting with the posterior ends of the rods. It is possible that they continue anteriorly parallel and adjacent to the stouter differentiation, but it may be true that the impregnating structure is a modification of the adherent part of the flagellum itself. The sharpness of differentiation of the V's and rods, and their constancy in form, indicates that they constitute definite structures, and are not to be explained as the results of capricious silver deposition. In favorably stained iron-haematoxylin preparations it was possible to find rods and V's of the same form as those demonstrated by the silver technique (pl. 32, *i*), the only difference being that they appeared more slender.

We have not observed blepharoplasts of granule form in which the flagella terminate, although these were clearly seen in other species of flagellates on the same slides. In the proximal parts of the flagella the only structures seen, either in iron-haematoxylin or silver preparations, were the rods and V's described above. Further studies of the flagellate may reveal more details of structure, as the existence of blepharoplasts and an integration of the flagellar organization is to be expected.

The axial structure in *Hexamitus pulcher* appears as though it were a single axostyle, extended posteriorly in a caudal point (pl. 32, *a, d*). The structure may be stained deeply and solidly, as is shown in the figures by Becker and Sassuchin. When the stain is not so heavy, the character of the pointed projection can be seen more clearly, extended into a pointed protuberance of the cytosome (pl. 32, *b, c*). In iron-haematoxylin preparations the rodlike axial structure is bordered by deep-staining, rather stout filaments, which begin anteriorly in the vicinity of the nucleus (pl. 32, *b;* pl. 33, *h*). These do not parallel the axial rod in a strictly longitudinal direction, but are somewhat twisted around it. The stainable stout filaments terminate posteriorly at the sides of the pointed end of the axial rod, and from these points there continue the caudal flagella. The stainable filaments are no doubt the intracytoplasmic

parts of the flagella, which are thickened or related to some stainable cytoplasmic structure. These intracytoplasmic flagella, however, apparently do not account for the entire axial structure of this flagellate.

At the posterior end of the body, somewhat anterior to the pointed posterior projection, the axonemes of the flagella diverge, so that there is a spearhead form in optical section (pl. 32, *c*, *h*). At the broadest part, which is the place of emergence of the flagella, silver impregnation shows what appears to be a ring (pl. 32, *a*). The caudal flagella have a length of 5.7–7.5$\mu$.

Anterior to the nuclei, at the apex of the body, silver impregnation shows a caplike structure (pl. 32, *a*, *d*).

The nuclei are ellipsoid in shape with a length of about 1.5$\mu$ and a width of about half this (pl. 32, *d*; pl. 33, *g*). Their anterior ends are close together near the anterior end of the body. Their longitudinal axes diverge, so that the nuclei remain in contact with the sides of the rounded anterior surface of the body. The nuclei appear quite empty of stainable chromatin, but there is a rounded body, which stains deeply with iron-haematoxylin, at the posterior end of each one (pl. 33, *g*). This is evidently an endosome.

Becker reported the presence in the cytoplasm of numerous spherules which he was able to stain with Janus Green. Our studies did not include living material stained *intra vitam*, and because the spherules often are either absent or are not stained after fixation we did not find them regularly. They were present in the material impregnated with protargol, which had been fixed in Hollande's fluid (pl. 32, *d*), indicating that probably it is the method of demonstration after fixation, rather than the dissolving action of the fixative, which accounts for the failure to find them in some material. In a series of slides from *Citellus beldingi* fixed in Bouin's fluid, the cytoplasm of *Hexamitus pulcher* contained many of these spherules deep-stained by iron-haematoxylin (pl. 33, *h*).

Before entering upon a comparative account of *Hexamitus pulcher* in relation to other species, it is desirable to give a statement in explanation of our spelling of the generic name. The original spelling by Dujardin, 1838, was *Hexamita*. The spelling *Hexamitus* is attributed in Neave's Nomenclator Zoologicus to Doflein, 1932; but in both author and date that is an error. *Hexamitus* was used by Bütschli (1884); was adopted by Klebs (1893); was used by Wenyon (1907); and was employed in Doflein's work of 1901 as well as in various editions of the Lehrbuch der Protozoenkunde. The word is obviously derived from the Greek $\mu\iota\tau o\varsigma$, and *-mita* is not an acceptable transliteration. We believe that in agreement with article 19 of the International Rules of Zoological Nomenclature the correction is justified.

*Hexamitus pulcher* belongs to the group of flagellates for which Lavier (1936*a*) proposed the generic name *Syndyomita*. In that genus, as he defined it, the intracytoplasmic structures related to the posterior flagella are close together and parallel, adherent, or fused in the median longitudinal axis of the body. Lavier gave the name *Syndyomita neglecta* to a flagellate of amphibia. He remarked, however, that the generic characteristics of the flagellate are found also in the flagellate of the rat which Prowazek (1904) described as

*Octomitus intestinalis*. He thought that confusion would be avoided by not using Prowazek's generic name for it, because this had not been defined and had been applied indiscriminately to various flagellates of the *Hexamita* type. If there is to be a generic distinction within the *Hexamitus* group of flagellates, *Octomitus* with type *intestinalis* has priority over *Syndyomita* with type *neglecta*, if the rat and amphibian flagellates have the same generic characteristics.

The flagellates of the *Hexamitus* group differ among themselves in important particulars, and it is probable that use of silver techniques will bring out still other differences than those that have been reported. No clear-cut differentiation can yet be made in groups of characteristics that might be used to allocate the flagellates to different genera. It seems preferable at present, therefore, to continue to use the generic name *Hexamitus* for all the flagellates of the series.

Wenrich (1930) reported *Hexamitus pulcher* Becker from the albino and Norway rat, the house mouse, a field mouse, and a ground squirrel at Flagstaff, Arizona. He stated that what appears to be the same species occurs in various amphibia. The flagellate in amphibia to which he referred is probably the one that was given the name *Syndyomita neglecta* by Lavier (1936*a*). Wenyon (1926) published drawings of what appears to be the same flagellate under the name *Hexamita intestinalis* Dujardin. Alexeieff (1911) described a flagellate of the same sort from amphibia under the name *Octomitus intestinalis* Prowazek, considering it the same as the one in the rat. The species of *Hexamitus* that occurs in salmonid fish, recorded as *Urophagus intestinalis* by Moroff (1903), as *Octomitus intestinalis truttae* by Schmidt (1920), and as *Octomitus salmonis* by Moore (1923) and Davis (1926) also appears similar to *Syndyomita neglecta* of Lavier. Moroff and Schmidt thought that they were studying the same flagellate that Dujardin (1841) reported from batrachians as *Hexamita intestinalis*. Dujardin's brief account does not indicate definitely which of the amphibian flagellates he designated by that name. Lavier (1936*a*) retained the name *Hexamita intestinalis* for a flagellate of amphibia which has rounded nuclei and widely separated intracytoplasmic flagellar structures. In the genus *Hexamitus*, the name *intestinalis* is unavailable for the mammalian flagellate, hence the name *Hexamitus pulcher* for the one originally called *Octomitus intestinalis*. *Hexamitus* species reported from Brazilian birds by Cunha and Muniz (1925, 1927) as *Octomitus acuminatus* and *O. elongatus* have the same type of axial structures. *Hexamitus marmotae* Crouch, 1934, from the caecum of *Marmota monax* may be the same species as the one here described in ground squirrels and other rodents. An account of the flagellate in the Norway rat, under the name *Syndyomita intestinalis* (Prowazek), was given by Morénas (1938).

In some species of *Hexamitus* the anterior flagella are shown as becoming free close to their origin in separate small granules anterior to the nucleus, as was well shown in *Hexamitus gigas* of the horse leech by Bishop (1933). Lavier (1936*a*) indicated this sort of origin of the flagella in his *Syndyomita neglecta* and he also (1936*b*) showed the flagella becoming free directly at

the anterior end in species of *Hexamitus* from marine fish. That mode of origin is not true of *Hexamitus pulcher*, and it appears also not to be true of the *Hexamitus* of salmonid fish.

In *Hexamitus salmonis*, Davis (1926) described the origin of the anterior flagella in a manner very similar to that we have reported in *Hexamitus pulcher*. The flagella of *H. salmonis* originate apically, pass for a distance along the surface of the body, and become free at the sides. Davis stated that "surrounding the base of each flagellum is a deeply staining layer, which extends from the axostyles to the point at which the flagellum leaves the body. Owing to this layer, it is often very difficult, if not impossible, to distinguish a definite granule at the base of the flagella, and one gets the impression that the blepharoplast complex is formed by the greater development of the deeply staining layer at the junction of flagella and axostyles." Davis's figures, especially the one of an apical view, show these rods along the basal parts of the flagella in an arrangement very much like that we have seen in *Hexamitus pulcher*, except that all three rods on each side are shown as meeting, instead of one of them being separate. This could not easily be established in iron-haematoxylin material; silver impregnation aids in arriving at the correct interpretation.

*Hexamitus salmonis* is a flagellate of larger size than *H. pulcher*, and both the anterior and caudal flagella appear in the published figures to be considerably longer.

The origin of the anterior flagella of *H. marmotae*, as described by Crouch (1934), is apparently very different from that which we have observed in the ground squirrel flagellate, but there may be a closer similarity than the figures indicate. Crouch found at the anterior margin of the body two blepharoplasts about a micron apart, each giving rise to one anteriorly directed flagellum; and at the base of the nuclei two granules, each of which gave rise to two flagella passing posteriorly. In the figures, the two posterior granules correspond exactly in position, size, and shape to the endosomes in the nuclei of *H. pulcher*. It is probable that in *H. marmotae* the actual origin of the flagella, which may really correspond to that described here in *H. pulcher*, was not seen. It is not unlikely that all of the flagella are directed posteriorly in the woodchuck species, the two shown by Crouch as anterior being accidentally turned forward from the other side of the body.

The flagella of *Hexamitus pulcher*, as shown by Becker (1926) and Wenrich (1930) are directed more or less posteriorly, or extend laterally from the margins of the body, but the regions of adherence to the surface of the anterior part of the body were not described. The description of flagellar origin in "*Syndyomita intestinalis*" given by Morénas (1938), might have been derived from partial observation of a structure like that we have described in *Hexamitus pulcher*.

In his original description of a flagellate from the intestine of the rat as *Oktomitus intestinalis*, Prowazek (1904) showed the flagella originating from sharply defined basal granules, four of which occupy positions corresponding to those of the upper ends of the rods in *H. pulcher*, whereas two are at the

posterior level of the nuclei and correspond more nearly to the posterior ends of certain of the rods. The flagella shown by Prowazek are distributed around the body in a symmetrical manner, not grouped four on one side as Crouch showed in *Hexamitus marmotae,* and the figure indicates the possibility of adherent anterior parts as in *Hexamitus pulcher.*

The pointed posterior projection, which resembles the terminal part of a trichomonad axostyle, was shown clearly by Prowazek in his original figure, and was clearly represented by Becker (1926) and Wenrich (1930, 1935). It was not shown, however, by Crouch (1934), who represented only the diverging lateral structures from which the flagella take origin. The structure of the posterior end of *Hexamitus salmonis,* with the lateral funnel-shaped chromatic structures (Davis, 1926), appears to be quite different from that of *Hexamitus pulcher.*

## Taxonomic Summary

### Hexamitus pulcher Becker, 1926

*Octomitus intestinalis* Prowazek, 1904, Arb. GesundhAmt., Berl., 21: 38.

*Octomitus intestinalis* Prowazek, 1904, Arb. GesundhAmt., Berl., 21: 41, pl. 4, fig. 107.

*Hexamitus,* larger form found in caecum, Wenyon, 1907, Arch. Protistenk., Supp. 1: 193, pl. 11, figs. 18, 19, 22, 23; pl. 12, fig. 35.

*Syndyomita intestinalis* (Prowazek, 1904), Morénas, Ann. Univ. Lyon (3), fasc. 1: 64, fig. 11b.

*Hexamitus pulcher* Becker, 1926, Biol. Bull., 51: 295, pl. 1, figs. 20–21.

*Type host.*—*Citellus tridecemlineatus tridecemlineatus* (Mitchill). Iowa. (Becker, 1926.) Xenosyntype slides GP-690: 1, 5, 17; 692: 15.

*Additional hosts.*—(Mammals only; amphibian hosts given by some authors are not listed here.)

Albino and Norway rat, house mouse, and field mouse (*Peromyscus leucopus*). (Wenrich, 1930.)

Rat (*Rattus norvegicus*) (Prowazek, 1904; Morénas, 1938).

*Citellus pygmaeus* Pallas. Kazakstan, R. S. F. Soviet Republic. (Sassuchin, 1931.)

*Citellus beecheyi beecheyi* (Richardson). Hastings Nat. Hist. Res., Monterey Co., Calif. Homocosyntype slides GP-681: 6, 7, 17, 20.

*Citellus beldingi beldingi* (Merriam). Reds Meadow, Madera Co., Calif. Homocosyntype slides GP-1163: 5; 1161: 2.

*Diagnosis.*—Length 6.3–10.3$\mu$, average 8.6$\mu$; width 3.4–6.3$\mu$, average 4.6$\mu$; six anterior flagella adherent anterolaterally along or in rodlike structures 1.7–3.4$\mu$ long, two pairs meeting in V's, free at posterior end of rods, length of free flagella 5–8$\mu$; axial structure present, extended posteriorly in caudal point, bordered by deep-staining, twisted, stout filaments that constitute the intracytoplasmic parts of the caudal flagella; length of caudal flagella 5.7–7.5$\mu$, two ellipsoid nuclei anterior, about 1.5 × 0.75$\mu$, anterior ends in contact, axes diverging obliquely posteriorly, endosome posterior in position, relatively large spherules often present in cytoplasm.

## Hexamitus teres n. sp.

(Pl. 33, *a–f*)

A species of *Hexamitus* that differs greatly from *Hexamitus pulcher* was found present in small or moderate numbers in the caecum of *Citellus tridecemlineatus, C. beecheyi,* and *C. beldingi.* It was not seen in the golden-

mantled ground squirrels. It resembles *Hexamitus muris* (Grassi), the species that commonly occurs in the small intestine of rodents, in that the intracytoplasmic longitudinal structures which terminate in the posterior flagella are widely separated in the cytoplasm. A search for *Hexamitus* was not made in the small intestine of the ground squirrels, but no flagellates of that genus were seen in the course of inspection for *Giardia*. It seemed possible that these flagellates in the caecum might be part of a population that is also represented, perhaps more abundantly, in the small intestine. The specimens studied in the caecum, however, differ so greatly from the published descriptions of *Hexamitus muris* that it is impossible to assign them to that species. The trivial name *teres* has been given to the species (Latin *teres*, rounded, polished, well turned) because of the rounded form of the body, as well as of the nuclei, together with the graceful form of the axial structures. Becker (1926) probably saw this flagellate in Iowa ground squirrels. He mentioned a species of *Hexamitus* or *Urophagus* which was very scarce in the caecum.

*Hexamitus teres* in *Citellus tridecemlineatus* ranged in length from 7.1–9.4$\mu$, averaging 7 9$\mu$; and in width from 4.7-7.1$\mu$, averaging 5.5$\mu$. The body is broadly rounded anteriorly and posteriorly (pl. 33, *a, c*) and lacks the posterior, median, pointed projection which is characteristic of *Hexamitus pulcher*. The nuclei are situated very close to the anterior surface of the body and to each other (pl. 33, *a, b*). They are regularly spherical in form and have a diameter of about 1.6$\mu$. There is a relatively large, spherical, central endosome, and the region between the endosome and the nuclear membrane, in the specimens examined, was without chromatic material.

As in *Hexamitus pulcher*, the anterior flagella adhere to the surface of the body in the first part of their course, and become free anterolaterally (pl. 33, *f*). In their normal position they are directed posteriorly. In the region where they adhere to the surface, there appear to be rodlike structures, 2.4–3.1$\mu$ long, that impregnate with silver and that also may be stained by iron-haematoxylin. The appearance following use of the two techniques is much the same. The rodlike structures are in two groups of three, and in each group they converge anteriorly and appear to meet (pl. 33, *d, e*). Their shape and relationship to one another is very much like that shown by Davis in *Hexamitus salmonis*. It was not possible in our specimens to make out a separation of an isolated rod and a V, as in *Hexamitus pulcher*. Each of the two groups of rods diverges at a right angle to the axis connecting the centers of the two nuclei. The meeting place of the proximal ends of the rods is just anterior to the nuclei in the region where those structures are adjacent. The free flagella extend from the ends of the rods, and have a length of 6.5–9$\mu$. As in *Hexamitus pulcher*, it is impossible to tell whether the rod is a separate structure along which the flagellum extends, or is a thickened part of the structure of the flagellum itself. The latter explanation is more likely.

In *Hexamitus teres* there is no structure in the median longitudinal axis of the body. The two longitudinal structures are situated laterally in the cytoplasm (pl. 33, *a, c, f*). They originate anteriorly near the place where the flagellar rods meet, anterior to the nuclei. In its proximal part, the plane of

the two longitudinal structures is more or less perpendicular to the plane of the two nuclei. The structures extend to the posterior end of the body, and in their course they are twisted (pl. 33, *f*). They have a narrow tube form for most of their length. In their distal part they increase in diameter, and finally flare rather abruptly, giving a structure that has a shape similar to that of the end portion of a trombone. These ends are flush with the surface of the cytosome, and are circular as seen in end view.

The posterior flagella can be seen in favorable haematoxylin-stained specimens of *Hexamitus teres* extending along a wall of the posterior, widened part of the apparent tubes, then becoming free at the terminus of the cytoplasm (pl. 33, *a*). They never appear to lie free within a widened tube, in the manner of a clapper within a bell. Their position may perhaps be compared to that of a clapper which lies close against one side of the bell; but it is uncertain whether the flagellum may not be applied to the exterior surface of the structure rather than being within a tube. The free parts of the posterior flagella have a length of 8–12$\mu$.

In several species of *Hexamitus* in marine fish, Lavier (1936*b*) reported the existence of a "manchon," a muff or mantle, surrounding each posterior flagellum in the cytoplasm. In its posterior part, the manchon was shown as expanded in a fosette, from which the flagellum emerged. In some of Lavier's drawings, the flagellum is represented along the whole length of the manchon posterior to the nuclei. In some of the species of *Hexamitus* from fish, the fosettes are situated a short distance anterior to the posterior end of the body. The structures related to the intracytoplasmic path of the flagella in those flagellates appear similar to the structures in *H. teres*, in which, however, the fosettes are close to the posterior terminus of the body.

In *Hexamitus salmonis*, Davis (1926) described a funnel-shaped development of the posterior part of the longitudinal intracytoplasmic structures, and observed that in end view these are circular in form. In the trout species the posterior flagella appear to lie in contact with the wall of the funnel toward the median axis of the body.

In *Hexamitus muris* the body has been described as very slender in shape. Wenrich (1930) gave the length as 7–9$\mu$, and the width as 2–3$\mu$. The length is about equal to that of *H. teres*, but the width is less than half of that in the species from *Citellus*. In our examinations we did not observe any specimens so narrow as even the broadest *H. muris* described by him. In *H. muris* the longitudinal structures, which generally appear to diverge more than they do in *H. teres*, are represented as fibrils of uniform diameter throughout the intracytoplasmic portion, with no posterior expansion, funnel, or fosette.

When Becker stated that this flagellate is a species of *Hexamitus* or *Urophagus*, he presumably had reference to the separation of the longitudinal structures related to the flagella that emerge posteriorly, and to the funnel-like or widened differentiations at the places of emergence. This type of organization, with a varying degree of development of the funnel-shaped structures, characterizes a group of species of *Hexamitus*. *Urophagus* is, as Lavier (1936*a*) remarked, a synonym of *Hexamitus;* being based on a morphological

disposition which exists in varying degree in *Hexamitus* species of that group. *Hexamitus teres* corresponds in general type to the amphibian flagellate for which Lavier (1936*a*) retained the name *Hexamita intestinalis*, and which he made representative of his restricted genus *Hexamita*. The amphibian flagellates of this nature were designated *Hexamita* sp. by Wenrich (1935); he used the name *Hexamita intestinalis* (?) for the flagellate of amphibia for which Lavier (1936*a*) subsequently created the new genus *Spironucleus*. *Hexamitus muris* and *H. teres* are representatives in rodents of Lavier's restricted genus *Hexamita*; also in the group may belong the species in monkeys described by Wenrich (1933).

## Taxonomic Summary

### Hexamitus teres n. sp.

*Type host.*—*Citellus tridecemlineatus tridecemlineatus* (Mitchill). Michigan. Museum Vert. Zool., Univ. Calif. 96798 ♂. Syntype slides GP-692: 1, 12, 15, 17.

*Additional hosts*—

*Citellus beldingi beldingi* (Merriam). Reds Meadow, Madera Co., Calif. Homoeosyntype slide GP-1164: 3.

*Citellus beecheyi beecheyi* (Richardson). Hastings Nat. Hist. Res., Monterey Co., Calif. Homoeosyntype slides GP-681: 8, 13, 18.

*Diagnosis.*—Length 7.1–9.4$\mu$, average 7.0$\mu$; width 4.7–7.1$\mu$, average 5.5$\mu$; body broad and rounded anteriorly and posteriorly; six anterior flagella adherent anterolaterally along or in rods 2.4–3.1$\mu$ long, which meet anteriorly in two groups of three; length of free anterior flagella 6.5–9$\mu$; no median axial structure, longitudinal structures separated in body, narrow tubular form flaring posteriorly in funnel form, caudal flagella continuing from funnel are 8–12$\mu$ long; two anterior spherical nuclei about 1.6$\mu$ in diameter, with relatively large spherical central endosome; no cytoplasmic spherules seen.

## Hexamastix muris (Wenrich)

(Pl. 34, *a–i*)

Becker (1926) recorded an infection in all except one of twenty *Citellus tridecemlineatus* by a flagellate that he named *Tetratrichomastix citelli*. He reported that the flagellate has four anterior flagella and a trailing flagellum. In our studies of the caecal flagellates of the same host species, we have found none with the flagellar complement he recorded; but there is present a species with five anterior flagella and a recurrent flagellum. That flagellate, which is a species of *Hexamastix*, is evidently the same as the one named *Tetratrichomastix citelli*.

Wenrich (1924) described, in a new genus and species, a caecal flagellate of rats as *Pentatrichomastix muris*. The flagellate possessed five anterior flagella and a trailing flagellum, and in 1930 Wenrich assigned it to the genus *Hexamastix*. Its host was the black house rat, *Rattus rattus*. Chang (1935*a*) recorded *Hexamastix muris* in two of eleven house rats, which belonged to the species *Rattus norvegicus*.

Wenrich examined our preparations of the flagellate in ground squirrels, and informed us that it resembles *Hexamastix muris;* he had remarked in

1930 on the similarity between that flagellate in the wild black rat and the one Becker described. He also stated to us that he had found *Hexamastix* in guinea pigs and marmots. We have found a flagellate morphologically identical with the ground squirrel *Hexamastix* in *Perognathus californicus*.

Sassuchin (1931) reported *Tetratrichomastix citelli* in *Citellus pygmaeus*. He found it in 186 of 242 animals; but it is apparent from his figures that he dealt at least in part with *Monocercomonoides*.

The flagellate was present in small numbers in our preparations made from *Citellus tridecemlineatus*. It was not found on our slides made from *Citellus beecheyi;* but it was seen, in small numbers, in the course of dark-field examinations of living material from that species, made at the Hastings Natural History Reservation. It was present in moderate abundance, along with *Monocercomonoides pilleata*, in a golden-mantled ground squirrel taken near the top of Yosemite Falls, but was not found in the other preparations from this host species. It was also moderately numerous, along with *M. pilleata*, in a specimen of *Citellus beldingi* taken at Tuolumne Meadows; but was not found in the other ground squirrels of that species. The studies made in preparation of this paper were drawn mainly from flagellates in *Citellus lateralis chrysodeirus*.

Measurements of twenty-five specimens from *C. lateralis chrysodeirus* gave a range in length from 6.3–10.2$\mu$, a range in width from 4.7–6.3$\mu$, and an average of 8.7$\mu \times$ 5.5$\mu$. The axostyle projects for a distance of about 1.6–3.9$\mu$, averaging about 2.8$\mu$. These dimensions compare well with the length of 7–13$\mu$, with an axostyle projection of 2–4$\mu$, that Becker (1926) reported.

In observations made on living material from *C. beecheyi* and *C. tridecemlineatus*, the grouping and activity of the flagella was observed to be very different from that of *Monocercomonoides pilleata*. The anterior flagella are united together in their proximal part in a whip, and are moved together. The whip is thrown forward, in the usual manner of the anterior flagella of a trichomonad; and then they move backward toward the surface of the body, separating from one another to a large extent. After the flagella are all turned backward, there is a momentary rest, then the whip is thrown forward again.

In fixed material the five anterior flagella are sometimes united proximally in a columnar grouping, but are often separated to the base (pl. 34, *a–i*). Some of them may be united in groups of two, three, or four. When flagella are united for their whole length, counts are difficult and may be made wrongly. But in silver preparations the deeply impregnating terminal granule or rod (pl. 34, *a, i*) often makes it possible to distinguish the individual flagella in a group. In this way, specimens which seemed at first to have less than five flagella were found actually to have five. A few specimens were seen with only three or four anterior flagella. Perhaps this reduction in number may have been the consequence of loss of certain flagella originally present.

The five anterior flagella range in length from 7.9–11.8$\mu$, and the recurrent flagellum has about the same thickness and length. In silver preparations the anterior flagella appear a good deal more slender than those of *Monocercomonoides pilleata*. They end in deeply impregnating knobs or rods, like the

flagella of *Pentatrichomonas hominis* and other trichomonads (pl. 36, *a, i*). The recurrent flagellum, also as in many other trichomonads, ends in a short slender filament (pl. 34, *a*).

The pelta is a conspicuous and sharply defined membranous structure at the anterior end of the body (pl. 34, *a–i*). In silver-impregnated preparations it appears black when seen in optical section (pl. 34, *b*). The membrane is curved around the anterior part of the body, with its plane flush with the surface of the body (pl. 34, *c f*). It has somewhat of a lunate form. One end is close to the blepharoplast (pl. 34, *b, g*) and the group of anterior flagella here extends forward from within the curve of the pelta. The recurrent flagellum passes posteriorly from the same region. The end of the pelta near the blepharoplast may be designated as the dorsal end. The ventral end extends to a point (pl. 34, *f*), but neither end is prolonged in a filament.

In its part just posterior to the nucleus the trunk of the axostyle (pl. 34, *a, i*) has a diameter of about 0.5$\mu$. As it passes anteriorly against the membrane of the nucleus, the axostyle is broadened in a spatulate form (pl. 34, *h, i*); it attains a width in the capitulum that is about three times the width of the trunk. The trunk of the axostyle tapers gradually posteriorly and may end close to the posterior end of the cytosome or extend up to a distance of several microns beyond the posterior end of the body. Its end is pointed, but it is not extended in a filament.

The nucleus has a size of about 2.4–3.9$\mu$ × 1.6–2.4$\mu$. It is elongated in a longitudinal direction. Its structure is that typical of a trichomonad. The interior is filled with finely granular chromatic material, and there is a small peripheral endosome that stains deeply with iron-haematoxylin and impregnates with silver.

The parabasal body (pl. 34, *a, b, i*) lies dorsal to the nucleus and in contact with it in its anterior part. It is a straight rod. In our silver preparations it was not impregnated well. Usually a simple filament was all that could be seen of it, but in some specimens it appeared as a stouter body. In its usual length it reached almost to the posterior end of the nucleus, but it was often shorter and sometimes a little longer than this.

Some details of the morphology of *Hexamastix muris* of rats are not known, so that a complete comparison with it of the ground squirrel flagellate has not been possible. The taxonomic assignment is therefore tentative. It is based on the probability that this flagellate in *Citellus* and *Perognathus* is the same as the one in rats and certain other rodents, just as *Hexamitus pulcher* and *Tritrichomonas muris* are common to various rodent species.

## Taxonomic Summary

**Hexamastix muris** (Wenrich, 1924)

*Pentatrichomastix muris* Wenrich, 1924, Anat. Rec., 29: 118.
*Tetratrichomastix citelli* Becker, 1926, Biol. Bull., 51: 294.
*Hexamastix muris* (Wenrich) Wenrich, 1930 in Hegner and Andrews, Problems and Methods of Research in Protozoology; p. 129, 141, fig. 12, e.

*Type host.—Rattus rattus* (Linnaeus). (Wenrich, 1930.)

*Additional hosts*—

*Citellus tridecemlineatus tridecemlineatus* (Mitchill). Iowa (Becker, 1926). Michigan. Hyposyntype slides GP-689: 29, 58; GP-690: 33.

*Citellus lateralis chrysodeirus* (Merriam). Yosemite Nat. Park, California. Hyposyntype slides GP-1077: 4, 5, 7.

*Citellus pygmaeus* Pallas. Kazakstan, R. S. F. Soviet Republic. (Sussuchin, 1931.)

*Citellus beldingi beldingi* (Merriam). Yosemite Nat. Park, California. Hyposyntype slides GP-1073: 1, 4.

*Citellus beecheyi beecheyi* (Richardson). Hastings Nat. Hist. Res., Monterey County, Calif.

*Perognathus californicus* Merriam. Hastings Nat. Hist. Res., Monterey Co., Calif. Hyposyntype slides GP-1012: 16, 19.

*Diagnostic description* (from *Citellus lateralis chrysodeirus*).—Length 6.3–10.2$\mu$, average 8.7$\mu$; width 4.7–6.3$\mu$, average 5.5$\mu$; five anterior often united proximally in a columnar grouping, length 7.9–11.8$\mu$; recurrent flagellum of similar length; trunk of axostyle moderately slender, pointed posteriorly, projecting usually 1.6–3.9$\mu$, capitulum moderately broadened, flattened against nucleus; parabasal body a straight rod about the length of the nucleus; pelta a narrow membrane, pointed at each end, curved around the blepharoplast region; nucleus ellipsoidal, elongated longitudinally, 2.4–3.9$\mu$ × 1.6–2.4$\mu$.

## Tritrichomonas muris (Grassi)

(Pl. 37, *a–d*; pl. 36, *a–g*)

Becker (1926) reported *Trichomonas muris* var. *citelli* in three of twenty *Citellus tridecemlineatus*. He evidently found it to be rather numerous in those individual animals. We did not find it in four *C. tridecemlineatus* from Michigan. Sassuchin (1931) reported *Trichomonas muris* var. *citelli* in three of 242 *Citellus pygmaeus*, and in the infected animals it was present in large numbers. Both of these authors remarked on its close resemblance to *Trichomonas muris*, evidently as described in rats and mice. They gave no reason for differentiating it as a variety; presumably that was done by Becker on account of the host difference. Since we do not here recognize a host difference in itself to be a valid criterion for taxonomic differentiation, and are unable ourselves to make a morphological differentiation, we will consider the ground squirrel flagellate under the name *Tritrichomonas muris*.

We did not find *Tritrichomonas muris* in *Citellus tridecemlineatus*, *C. beecheyi*, or *Citellus beldingi*. In *Citellus lateralis chrysodeirus*, however, it is generally present as the dominant if not the only flagellate. Of nine golden-mantled ground squirrels taken near Rainbow Falls, Madera County, California, *T. muris* was present in large numbers in five. In three of the other four there were no flagellates; the fourth had some *Monocercomonoides pilleata*. Of two *C. l. chrysodeirus* taken near the top of Yosemite Falls, *T. muris* was present only in one, which lacked other flagellates. The other squirrel had *Monocercomonoides* and *Hexamastix*. Of nineteen samples of caecal contents of *C. l. bernardinus* sent from San Bernardino County, *T. muris* was present in all but three, and was numerous or very abundant in all except one of the sixteen. This flagellate is evidently as characteristic a part of the caecal fauna of golden-mantled ground squirrels as of rats and mice, but it is either absent or infrequent in the other species of ground squirrels examined.

All original information given in this paper about *Tritrichomonas muris* has been drawn from study of the flagellates in *Citellus lateralis chrysodeirus*.

In many specimens on our slides of this trichomonad of the golden-mantled ground squirrel the body is more or less bilaterally symmetrical in form except for the occurrence of the anterior part of the axostyle at one side of the nucleus. The undulating membrane and costa extend longitudinally along the region designated as dorsal, like an undulated dorsal fin; but the costa may be displaced to one side of the axostyle in its posterior part. In many other specimens the undulating membrane follows more or less of a spiral course in passing posteriorly, in a direction that is counterclockwise as viewed from the anterior end. There is sometimes a complete turn around the body, though more often there is only a half turn or less. These specimens are not symmetrical, and the designation dorsal has reference only to the position of the costa and membrane at their beginning.

The length of the flagellates ranged from 11.8$\mu$ to 19.8$\mu$; and the width from 4.3$\mu$ to 9.9$\mu$, averaging 16.1$\mu$ × 7.4$\mu$. No very small trichomonads were found in the golden-mantled ground squirrels. Those small forms sometimes accompany the larger ones in rodents, and by some have been regarded as small forms of the same species, and by others differentiated as *Trichomonas* or *Tritrichomonas minuta*. The axostyle projects posteriorly for a distance of 2.5–4.3$\mu$, averaging about 3$\mu$.

The three anterior flagella have a length of 6.8–9.9$\mu$, and are unusually slender, as seen in silver preparations, in comparison with such flagella as those of *Pentatrichomonas hominis*. Each of them ends in a deeply impregnating granule (pl. 35, *d*). The ends do not appear elongated and bent over as in some other trichomonads. Usually the three anterior flagella are separate from one another to their place of origin from the cytosome.

The marginal flagellum of the undulating membrane has a ribbon form (pl. 35, *a–d*). It narrows to a stout filament both anteriorly where it meets the blepharoplast, and posteriorly where it extends as a free flagellum for a distance of 9.3–14.9$\mu$ beyond the end of the membrane. In silver preparations it may be solidly impregnated as a band. The flagellum is directed edgewise to the body and is thrown into bold undulations (pl. 36, *b*). The free part of the trailing flagellum tapers in its first half, and in its posterior half remains about a uniform size, which is still much greater than that of an anterior flagellum (pl. 36, *b*). The flagellum ends bluntly; we did not observe an acroneme extension like that which exists in many trichomonads.

A sheet of protoplasm extends between the inner edge of the flagellum and the main body of cytoplasm, at the periphery of which the costa normally lies. In normal specimens the flagellum is adherent to the edge of this membrane; but in preparations it is often partly or completely detached (pl. 36, *a*). It may be torn off entirely, and it is sometimes detached from the body. This detachment from the membrane is evidently the result of mechanical influences in preparation, but its frequency of occurrence shows that the union of the edge of the flagellum with the edge of the membrane is not very firm.

In some silver-impregnated specimens the whole membrane is darkened; in

others it appears clear. The edge of the sheet of protoplasm is a filament (pl. 36, *a*). In most normally shaped specimens the membrane filament appears to be single, and comes into contact posteriorly with the posterior end of the costa. Especially in specimens that have been distorted by partial drying, it can be seen that the outer filament of the membrane is a structural entity. In its posterior part it may be displaced in relation to the costa, and it has an appearance similar to that of the posterior part of the costa. In some of these distorted specimens there are two parallel filaments, one exterior and one interior, at the outer margin of the membrane. These are particularly apparent along the middle part of the membrane, where they are sometimes very sharply defined.

The costa is in many preparations silver-impregnated or haematoxylin-stained as a homogeneous black structure. In other preparations the edges are impregnated or stained and the interior is more or less clear, like a narrow clear band down the middle. In the middle part of its length the costa is stout; in large specimens its width may be 0.5$\mu$. In its anterior five or six microns, and in its posterior part, the costa gradually tapers (pl. 35, *a, d*). It terminates in small ends, which are not filaments. At the posterior end the costa is usually more gradually and sharply pointed than at the anterior end. The costa is a rounded rod. Its shape is the same when seen at the upper edge of the body as when seen at the side.

At the place of meeting of the mastigont organelles there is a rounded body which in larger specimens has a diameter of about one micron (pl. 35, *a, c;* pl. 36, *b*). In our preparations we have not been able to find a heterogeneous or granular structure in this body. It is apparently a single large blepharoplast. No pelta has been found.

The organelles leave the blepharoplast spherule in definite positions (pl. 35, *a, d*). The anterior flagella meet its anterior part. The costa, after turning transversely in its course and sometimes running somewhat posteriorly, meets its posterior part. The trailing flagellum leaves the blepharoplast near the place of origin of the costa. Leaving the blepharoplast posteriorly near the origin of the costa is a filament which passes posteriorly toward the nucleus and along its dorsal edge.

The last structure is evidently the filament of the parabasal body. In some series of silver-impregnated flagellates this filament was all that could be seen; the main substance of the parabasal had failed to impregnate. In some specimens on the same slides, careful observation did reveal the parabasal substance, although it was very faint. In another series of silver preparations the whole parabasal was deeply impregnated. These differences in the reaction of the parabasal to the protargol process were probably the result of the use of different lots of protargol, though even with the same lot the results are not always predictable.

The parabasal body is a sausage-shaped structure with a thickness varying between about one-half and one micron (pl. 35, *d;* pl. 36, *d–g*). The parabasal body usually has a more or less uniform width except close to its place of attachment to the blepharoplast. Here there may be a small gap; the main

parabasal substance does not meet the blepharoplast, but the filament crosses the gap (pl. 36, *e–g*). In other specimens the main parabasal substance seems to come directly into contact with the blepharoplast (pl. 36, *a*). The parabasal body is rounded broadly at its posterior end. In some specimens it is thicker in its posterior part than more anteriorly (pl. 36, *d*), but this clavate form is not general in the material we have studied.

In its usual position the parabasal body passes straight posteriorly to near the anterior end of the nucleus, then curves dorsally and passes around the dorsal surface of the nucleus, lying close to the membrane (pl. 36, *a*). Contact with the nuclear membrane is usual, but sometimes the parabasal is more or less separated from the nucleus. It usually terminates near the posterior end of the nucleus, but it may be somewhat shorter or occasionally a little longer than this. The length of the parabasal body averages about 6.2$\mu$, ranging from 5$\mu$ to 7.4$\mu$, in the flagellates of larger size.

The size of the parabasal body did not vary a great deal in the series we studied, which was from one squirrel; it seldom extended beyond the posterior end of the nucleus. Wenrich (1921) showed the parabasal body of *T. muris* in mice to be much longer than this, extending for some length posterior to the nucleus. We have studied specimens of *T. muris* from laboratory rats and hamsters, as well as a number of wild rodents. Trichomonads prepared at the same time from two different laboratory rats showed differences in the development of the parabasal body. Those in flagellates from one rat were similar in size and position to those from the golden-mantled ground squirrel; few extended beyond the posterior end of the nucleus. Some of the flagellates from the other rat had similarly short parabasals, but in many the parabasals were longer, and often curved in a C form. The length of the part posterior to the nucleus was in some instances as much as the length of the nucleus. Often the terminal part was enlarged in a club form. A similar variability was observed in parabasals of flagellates from hamsters. Specimens of *Tritrichomonas muris* with similarly long parabasals have been seen in *Peromyscus boylei*, *Peromyscus truei*, *Dipodomys venustus*, *Neotoma fuscipes*, and a species of *Microtus*. In these wild rodents the longer parabasals are characteristic, but some specimens with shorter ones not longer than the nucleus occur. Because of these observations on the variability in size of the parabasal body, we do not consider the fact that the structure in the trichomonad of *Citellus* is shorter than that generally considered typical of *T. muris* to be of taxonomic significance.

In large specimens the trunk of the axostyle has a diameter of about 1.5$\mu$. It retains the same diameter from immediately posterior to the nucleus to the posterior end of the body. Shortly before it reaches the end of the cytosome it is surrounded by two rings (pl. 35, *a*; pl. 36, *b*). These rings may be demonstrated well in haematoxylin-stained specimens, but in protargol preparations they are not demonstrated (pl. 35, *d*). The terminal part of the axostyle, for a length of between 2.5 and 4.3 microns, projects from the body and is tapered in a conical form. The projecting part takes a black surface impregnation in protargol preparations, and there is a sharply defined circular margin at the

place where the axostyle leaves the cytoplasm (pl. 35, *d*). In the same specimens, the membrane of the trunk of the axostyle within the cytoplasm is faintly defined. It is evident in the material that the axostylar projection has no surrounding cytoplasm.

The trunk of the axostyle comes into proximity to the nucleus somewhat ventral to its posterior end (pl. 35, *d*). It continues along the ventral and right side of the nucleus, remaining in contact with that structure. There is no enlargement of the anterior part of the axostyle. As observed from its lateral aspect, it appears to narrow somewhat as it approaches the blepharoplast (pl. 36, *b*).

Parallel to the costa at a distance from it of about 0.5–0.75$\mu$ is a row of granules that stain deeply with iron-haematoxylin (pl. 35, *a–c*). The row begins at the blepharoplast, extends along the dorsal surface of the nucleus, parallels the costa, and is discontinued a few microns short of the posterior end of the costa (pl. 36, *b*). In some places the granules are in more than one row (pl. 35, *a*, *b*). In our material the granules were aggregated closely in a row that appeared continuous, and was often as sharply outlined as the costa itself.

A second linear group of granules extends along the anterior part of the axostyle (pl. 35, *c;* pl. 36, *b*). Posterior to the nucleus it lies along the dorsal side of the trunk of the axostyle. It may be closely applied to the axostyle for its whole length (pl. 36, *b*, *c*), but often it is separated in its posterior part (pl. 35, *a*, *c*). In its anterior part alongside of the nucleus the aggregate of granules is often broad. It is sometimes curved in a crescentic form covering the right side of the axostyle and often extended to a point ventral to the axostyle (pl. 35, *c*).

The rows of paracostal and para-axostylar granules are more or less parallel to one another and are in a rather precise location (pl. 36, *b*, *c*). When the trunk of the axostyle is bent away from its usual position, the para-axostylar row remains parallel to the paracostal row, instead of following the axostyle. The posterior part of the para-axostylar row sometimes turns away from the paracostal row, toward the axostyle (pl. 35, *c*).

The granules of these two rows are usually not demonstrated in silver preparations.

In the trichomonad from the golden-mantled ground squirrel the nucleus is elongated in form, measuring about 4.3–6.2$\mu$ × 1.9–3.1$\mu$, and averaging about 5$\mu$ × 2.5$\mu$. In some specimens it appears regularly ellipsoidal in shape, being equal in size in its anterior and posterior part. The usual shape is asymmetrical pyriform, smaller in the posterior part than in the anterior part, concave on the ventral side and convex on the dorsal side (pl. 35, *a*, *b*). When fixed and stained by iron-haematoxylin, the nucleus shows numerous granules which are moderately large and sometimes fairly widely spaced (pl. 35, *a*, *b*). In many of the preparations of the nuclei it was not possible to recognize an endosome. Some nuclei that are favorably differentiated show a body, or sometimes more than one body, which is deep-stained and surrounded by a clear zone (pl. 35, *d;* pl. 36, *c*). In some protargol preparations the nuclei are

not visible; in others the membrane and granules take a light impregnation, and the endosome may be deeply impregnated.

In his comparison of *T. muris* and *T. wenrichi*, Crouch (1936) stated that in the former species the nucleus is oval or broadly elliptical, as Wenrich (1921) had described it, whereas in the latter species it is pyriform. In the trichomonads in our preparations from a number of different rodents, the nucleus often has an ellipsoidal form; that shape appears to be more frequent in some populations than in others. In many specimens of *T. muris* from laboratory rats, we have observed nuclei of the same shape as the asymmetrically pyriform nuclei of the ground squirrel trichomonads. In the shape of the nucleus there is a degree of variability which precludes using it for taxonomic differentiation within the *T. muris* group, at least without careful and extensive comparison between strictly comparable preparations.

A parasitic organism similar to that which Crouch described as *Sphaerita trichomonadis* in *T. wenrichi* was found in many of the trichomonads from one ground squirrel (pl. 36, *b*). There are groups of spherules which are about one micron in diameter. Some are a little smaller and some larger than this, but they are not so large as those reported by Crouch. They may be stained densely by iron-haematoxylin; but when suitably differentiated a spherule shows the small black granule in a clear area which was described by Crouch as a nuclear spot. The spherules lie in a clear area and are not related to one another, not being imbedded in a surrounding protoplasm. The clear region in which they occur is more or less spherical, but is often misshapen by its confinement among cytosomal structures. The boundary of the clear region is the limit of the surrounding cytoplasm; there is no visible membrane.

Crouch referred to these groups of spherules in terms of plasmodia containing spores, which increase in number by division. The division of spherules suggests to us a solitary microörganism which multiplies, rather than *Sphaerita* in which in the early stages there is a plasmodium containing nuclei that multiply successively. The spores in *Sphaerita* occur at the end of the period of development; in the sporangia they are isolated bodies not imbedded in protoplasm, and they do not multiply as spores.

In a few of the flagellates there were found groups of granules, one-third micron or less in diameter, in a spherical, clear zone (pl. 36, *c*). These evidently represent a different parasite.

Trichomonads that have been described from the intestine of rodents are mainly of the *T. muris* type, but there are also other forms. Among the flagellates of the *T. muris* type, there has been no definite designation of taxonomic differences that justify the establishment of species in different hosts. Pending further study, it seems desirable to regard the trichomonads that are recorded from rodents and correspond morphologically to *T. muris* as members of that species. They cannot properly be differentiated, as was the variety *citelli*, solely on the basis of occurrence in a different rodent.

*Tritrichomonas caviae* of the guinea pig belongs to the *T. muris* group, and is similar to *T. muris* in many respects, although apparently differing significantly in the relatively greater length of the anterior flagella, and in the

distribution of cytoplasmic and axostylar granules. Valuable figures for comparison of *T. muris* and *T. caviae* may be observed in Wenrich, 1935, plate 4. Named by Davaine, 1875, *T. caviae* is the oldest species of the group.

*Tritrichomonas muris* (usually called *Trichomonas muris*) was first reported in rats and mice by Grassi (1879); in 1881 Grassi recorded flagellates of this sort in *Arvicola arvalis*, in addition to *Mus musculus* and *Mus rattus* ("una *Cimaenomonas* del pari similissima a quella dei Batraci"). It was also reported from *Mus rattus* by Galli-Valerio (1907) and has been recorded from laboratory rats and mice by various authors. *T. muris* was recorded from *Peromycus maniculatus gambeli* by Kofoid and Swezy (1915); from *Microtus arvalis* by Lavier (1921); from *Peromyscus leucopus* by Wenrich (1921); from *Apodemus sylvaticus*—besides *Epimys rattus, E. norvegicus,* and *Mus musculus*—by Morénas (1938). Designation of a variety, as *T. muris* var. *citelli,* for the flagellate found in *Citellus tridecemlineatus* by Becker (1926) and in *Citellus pygmaeus* by Sassuchin (1931) is not in keeping with taxonomic procedure in trichomonads, or in most other flagellates. It is desirable either to regard this flagellate as *Tritrichomonas muris,* as we have done, or to establish a full species *T. citelli.* The latter procedure could not be based on any sound diagnostic differences, so we have chosen the former course.

There is a close similarity to *T. muris* in *Trichomonas wenrichi,* described by Crouch (1933) from *Marmota monax* in Iowa and Kentucky. Crouch noted its similarity both to *T. muris* as described by Wenrich (1921) and to *T. muris* var. *citelli* as described by Becker (1926). Although he tabulated a series of morphological differences between *T. wenrichi* and *T. muris,* that table includes certain features of which the validity or the taxonomic value may be questioned. *T. wenrichi* is reported to attain a larger size than the ground squirrel trichomonads or those of rats and mice, but species of the family generally have a high degree of variability in size.

In reporting a species of *Trichomonas* from a porcupine, *Hystrix bengalensis,* Knowles and Das Gupta (1929) stated that it corresponds very closely in size and general morphology to *T. muris* and *T. caviae,* and recorded a maximum length of 26$\mu$ (although apparently in dried and distorted specimens). That size was exceeded by only 4 out of 100 *T. wenrichi* as reported by Crouch. They also stated their belief that the *Trichomonas* of rodents may all be one and the same organism.

The trichomonad reported from the muskrat, *Ondatra zibethica,* by Bishop (1934) resembles flagellates of the *T. muris* group in certain respects, but the three anterior flagella are stated to be as long as the body. Cytological details are not given.

Hegner and Ratcliffe (1927) described a trichomonad flagellate from the prairie dog, which belongs to the same tribe of rodents—the Marmotini—as *Marmota* and *Citellus.* According to their account, the flagellate is very much unlike *T. muris. Trichomonas cynomysi* has four long anterior flagella, a slender axostyle which stains deeply with iron-haematoxylin, and a spherical nucleus.

Morénas (1938) reported from *Pitymys subterraneus,* a member of the

tribe Microtini, a trichomonad with two anterior flagella, named *Ditrichomonas lavieri. Trichomonas myoxi,* described from *Myoxus quercinus* by Galli-Valerio (1927) also is stated to have two anterior flagella. Even if the number of anterior flagella actually is only two in these flagellates, they do not belong in the genus *Ditrichomonas.* The type species of that genus, *imnsi* from *Archotermopsis wroughtoni,* has four anterior flagella (Cleveland *et al.,* 1934), although the number was erroneously reported as two by Cutler, 1919.

Wenrich (1946) referred to the presence in rats and mice of *Trichomonas muris, Trichomonas minuta, Trichomonas wenyoni,* and *Trichomonas hominis. Pentatrichomonas hominis* is an organism of a very different type from the commonly occurring trichomonads of wild rodents. *Trichomonas minuta* is similar in general to *T. muris,* but is small in size, ranging from 3–10 microns, a lower size range also noted in *T. caviae.* (See Wenyon, 1926.) *Trichomonas wenyoni* (formerly known as *T. parva;* see Wenrich, 1930, 1946) is a small flagellate with three relatively long anterior flagella, and an undulating membrane not extending posterior to the middle of the body.

*Trichomonas guiarti,* described by Morénas (1938) from a white rat, is said to have usually three but exceptionally four anterior flagella, a large cytosome, and no axostyle. Its validity as a distinct species is doubtful.

This brief review of some reports of trichomonads in rodents indicates that it cannot be said that rodents have only one species, and it is not sound to assume that a new rodent trichomonad is necessarily likely to be *T. muris.* Nevertheless, where no clear-cut morphological difference from *T. muris* has been established, it is desirable to assign the flagellates to that species.

## Taxonomic Summary

### Tritrichomonas muris (Grassi)

*Monocercomonas muris* Grassi, 1879, Gazz. Med. Ital. Lombard., ser. 8, v. 1: 448.

*Cimaenomonas,* Grassi, 1881, Atti. Soc. Ital. Sci. Nat., 24: 158.

*Trichomonas intestinalis,* Wenyon, 1907, Arch. Protistenk., Supp. 1: 184, pl. 11, figs. 1–17, 20–21.

*Trichomonas muris* (Galli Valerio, 1907, Zbl. Bakt., I, 44: 529, fig.

*Trichomonas muris* (Hartmann), Hartmann, 1910, Praktikum der Protozoologie, p. 45, fig. 27.

*Trichomonas muris* (Hartmann) var. *citelli* Becker, 1926, Biol. Bull., 51: 293, pl. 1, figs. 13–15.

*Tritrichomonas muris* (Galli-Valerio, 1907), Wenrich 1930 in Hegner and Andrews, Problems and Methods of Research in Protozoology (N.Y., Macmillan), p. 140, fig. 12, a, b.

*Type host.—Mus musculus* Linnaeus. (Grassi, 1881.)

*Additional hosts—*

*Rattus rattus* (Linnaeus). (Grassi, 1881; Galli-Valerio, 1907.)
*Rattus norvegicus* (Erxleben). (Various authors.)
*Peromyscus maniculatus gambeli* (Baird). California. (Kofoid and Swezy, 1915.)
*Peromyscus leucopus* Rafinesque. Eastern U.S.A. (Wenrich, 1921.)
*Microtus arvalis* Pallas. Europe. (Grassi, 1881; Lavier, 1921.)
*Apodemus sylvaticus* (Linnaeus). Europe. (Morénas, 1938.)
*Citellus tridecemlineatus tridecemlineatus* (Mitchill). Iowa. (Becker, 1926.)
*Citellus pygmaeus* Pallas. Kazakstan, R. S. F. Soviet Republic. (Sassuchin, 1931.)

*Citellus lateralis chrysodeirus* (Merriam). California. Reds Meadow, Madera Co. Hyposyntype slides GP-1145: 4, 7, 12, 17; GP-1170: 2, 3, 4. Yosemite Nat. Park. Hyposyntype slides GP-1078: 27, 29, 31, 33.

*Diagnostic description*, from *Citellus lateralis chrysodeirus*. Length 16.1 (11.8–19.8) $\mu$; width 7.4 (4.3–9.9) $\mu$; three slender anterior flagella 6.8–9.9$\mu$ long; costa fairly stout, full length of body; marginal flagellum of undulating membrane ribbon-formed except anteriorly and posteriorly, free beyond end of undulating membrane for 9.3–14.9$\mu$; parabasal body elongate, curved against dorsal surface of nucleus, terminating often near posterior end of nucleus; trunk of axostyle fairly stout, conical posterior projection 2.5–4.3$\mu$ long, two rings around axostyle near place of projection; paracostal row of granules along most of length of costa; shorter para axostylar row of granules along the anterior part of the axostyle; nucleus 5 (4.3–6.2) $\mu \times 2.5$ (1.9–3.1) $\mu$, shape ellipsoidal or asymmetrical pyriform, concave on ventral and convex on dorsal side.

## LIST OF KNOWN FLAGELLATE INFECTIONS OF THE CAECUM OF SPECIES OF CITELLUS

*Citellus tridecemlineatus tridecemlineatus* (Mitchill). Iowa, Michigan
- *Chilomastix magna* Becker (Type host)
- *Monocercomonoides pilleata* Kirby & Honigberg (Type host)
- *Hexamitus pulcher* Becker (Type host)
- *Hexamitus teres* Kirby & Honigberg (Type host)
- *Hexamastix muris* (Wenrich)
- *Tritrichomonas muris* (Grassi)

*Citellus beecheyi beecheyi* (Richardson). California
- *Chilomastix magna* Becker
- *Monocercomonoides pilleata* Kirby & Honigberg
- *Hexamitus pulcher* Becker
- *Hexamitus teres* Kirby & Honigberg
- *Hexamastix muris* (Wenrich)

*Citellus pygmaeus* Pallas. R. S. F. Soviet Republic
- *Chilomastix magna* Becker
- Probably *Monocercomonoides pilleata* Kirby & Honigberg
- *Hexamitus pulcher* Becker
- *Hexamastix muris* Wenrich
- *Tritrichomonas muris* (Grassi)

*Citellus beldingi beldingi* (Merriam). California
- *Chilomastix magna* Becker
- *Monocercomonoides pilleata* Kirby & Honigberg
- *Hexamitus pulcher* Becker
- *Hexamitus teres* Kirby & Honigberg
- *Hexamastix muris* (Wenrich)

*Citellus lateralis chrysodeirus* (Merriam). California
- *Monocercomonoides pilleata* Kirby & Honigberg
- *Hexamastix muris* (Wenrich)
- *Tritrichomonas muris* (Grassi)

*Citellus lateralis bernardinus* (Merriam). California
- *Tritrichomonas muris* (Grassi)

## SUMMARY

An account is given of all species of flagellates that have been found in the caecum of *Citellus tridecemlineatus tridecemlineatus* of Iowa and Michigan and of three ground squirrels in California—*C. beecheyi beecheyi*, *C. beldingi beldingi*, and *C. lateralis chrysodeirus*. Two new species, for which *C. tride-*

*cemlineatus* is the type host, are described: *Monocercomonoides* **pilleata** and *Hexamitus* **teres.**

Excepting the Mantled Ground Squirrels, the flagellate population is similar in all the hosts and in *Citellus pygmaeus* of Russia. In *Citellus lateralis chrysodeirus* and *C. l. bernardinus* a number of species have not been found, although they may be present; but the chief difference is the frequent occurrence of large populations of *Tritrichomonas muris,* which has been found infrequently or not at all in the other hosts.

*Chilomastix magna* closely resembles *C. intestinalis* of the guinea pig. The three anterior flagella originate from separated basal granules, and there is a recurrent flagellum, about half as long as those, that passes posteriorly over the opening of the cytostome. The fibril that borders the cytostome continues around its anterior margin, instead of there being two fibrils that terminate separately in anterior granules. The fibril on the right of the cytostome turns in a loop and continues deep in the cytoplasm. There is no parabasal body.

*Monocercomonoides pilleata* possesses certain structures, demonstrable by silver protein impregnation, that have not hitherto been detected in the genus. The recurrent flagellum adheres to the surface above a rodlike peripheral structure. In the anterior region there is a curved membrane, designated as the pelta. The four flagella are all of the acroneme type, each terminating in a short filament. There is no parabasal body.

The six anterior flagella of *Hexamitus pulcher* are separately adherent to the body for a length of two or three microns in or along rodlike structures, two pairs of which appear to meet anteriorly in two V's. There is an axial structure, extended posteriorly in a pointed projection, along or in which are two filaments that constitute the intracytoplasmic parts of the caudal flagella.

*Hexamitus teres* has the six anterior flagella similarly adherent along or in rods that meet anteriorly in two groups of three. There is no median longitudinal structure. The two longitudinal structures are lateral to the median axis, and each has a narrow tubular form flaring to a posterior funnel from which a caudal flagellum extends. This flagellate is infrequent in the caecum.

*Hexamastix muris* has at the anterior end a narrow, curved, pointed membrane, the pelta, which has not hitherto been described in the genus. The five anterior flagella terminate anteriorly in rods or knobs, as in other trichomonads, and the recurrent flagellum is of the acroneme type. There is a rod-shaped parabasal body that terminates near the posterior end of the nucleus.

The structure of *Tritrichomonas muris* of *Citellus lateralis* agrees with that of the species in other rodents. The three anterior flagella are short and slender, terminating in silver-impregnating granules. The marginal flagellum of the undulating membrane appears to have a ribbon form in its adherent part. The free part terminates bluntly, not being an acroneme as is usual in trichomonads. No pelta is present.

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# PLATES

All figures have been made with the aid of the camera lucida.

Abbreviations for methods of preparation:

B. Bouin's fluid.

Ch. Champy's fluid.

Holl. Hollande's cupric picroformol.

S. Schaudinn's fluid.

Hematein. Mordant in 0.5 per cent alcoholic solution of iron alum; stain in ⅔ per cent alcoholic solution of hematein.

Heid. Heidenhain's iron-haematoxylin.

Prot. Impregnation by 1 per cent protargol, with copper wire immersed in solution, Bodian technique.

## PLATE 30

*Chilomastix magna* Becker

*a*, *c*–*o*. From *Citellus t. tridecemlineatus.*

*b*. From *Citellus b. beecheyi.*

*a*. Diagram showing the anterior flagella, the cytostome, and the recurrent flagellum, which is turned upward. Ch. Heid. × 3,000.

*b*. Diagram showing the cytostome bordered by a continuous fibril, with a dark area along its right margin; the nucleus; and the three anterior flagella originating from well-separated granules. The recurrent flagellum is not shown. Holl. Heid. × 2,400.

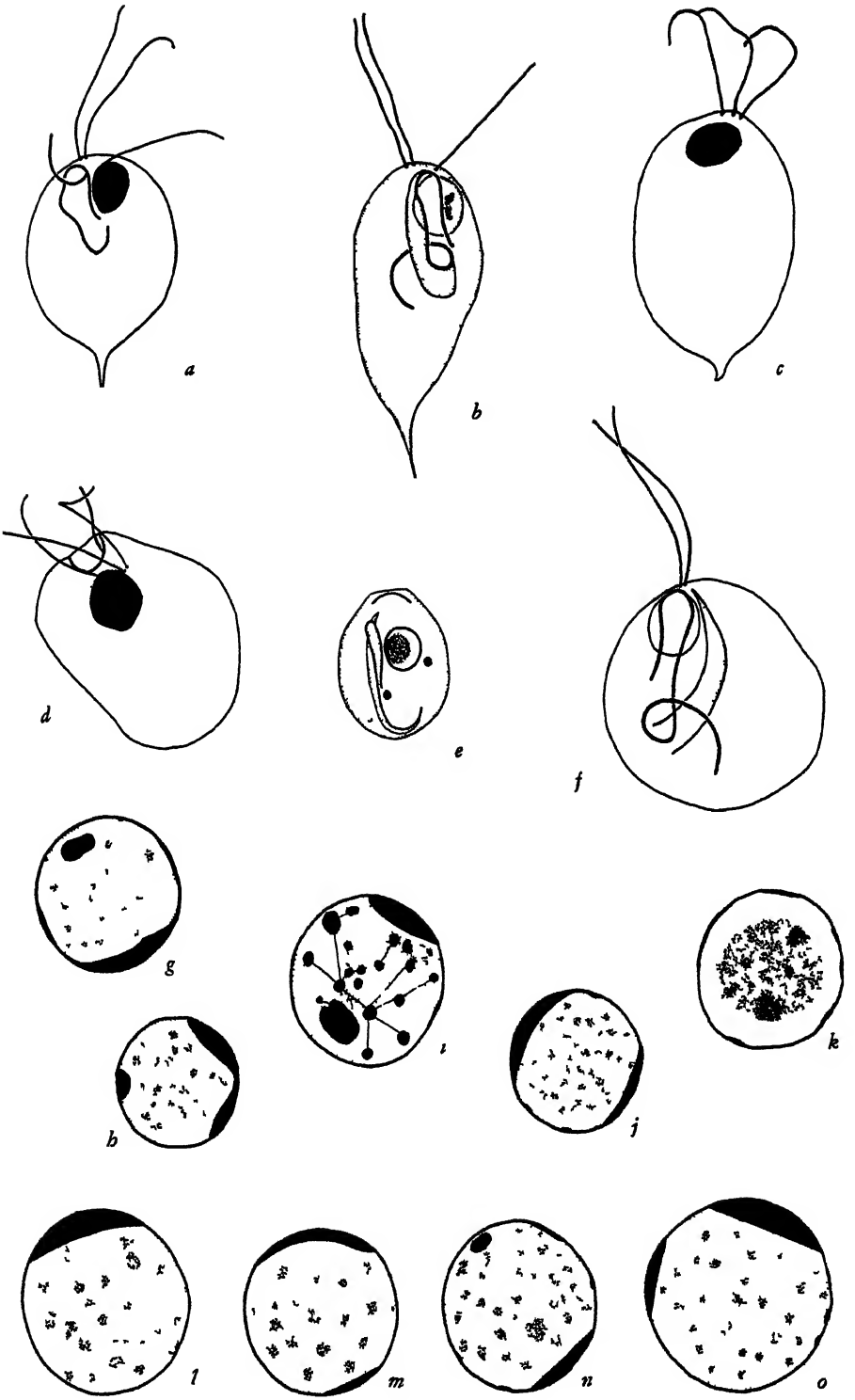
*c*. Diagram showing the arrangement of the anterior flagella. Ch. Heid. × 3,000.

*d*. Diagram showing the upper part of the cytostome from the anterior end of the flagellate. The three anterior flagella are shown, also the recurrent flagellum extending free from within the anterior margin of the cytostome. Ch. Heid. × 3,000.

*e*. Cyst, showing the arrangement of the cytostomal fibril. S. Heid. × 2,830.

*f*. Diagram showing the cytostomal fibril, which here lies on the under-surface of the body, curved around the anterior margin of the cytostome and looped posteriorly. Also represented is the dark cytoplasmic differentiation which has inappropriately been referred to as the parabasal body. Holl. Heid. × 3,000.

*g*–*o*. Nuclei showing the arrangement of the inner chromatin and the deep-staining plaques of peripheral chromatin Figs. *m*, *o*, Holl. Prot.; others, S. Heid. × 7,200.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## PLATE 31

*Monocercomonoides pilleata* n. sp.

*a–e, g, h.* From *Citellus b. beecheyi.*

*d, f.* From *Citellus t. tridecemlineatus.*

*a.* Diagram showing the nucleus with central endosome; the slender stained axostyle surrounded at the posterior end of the cytoplasm by an axostylar ring; the two pairs of flagella, one of the four being recurrent; and the filament connecting the two granules from which the flagella originate. Holl. Heid. × 3,600.

*b.* Silver-impregnated specimen showing the two pairs of flagella, all of which terminate in filaments and one of which is recurrent and adherent; the rod along the region of adherence of the recurrent flagellum; the axostyle and axostylar ring; and an outline of the curved, pointed pelta. Nucleus omitted. Holl. Prot. × 3,600.

*c.* Diagram of the mastigont structures in the anterior part of the body, showing the curved pelta tapering at one end to a point continued in a filament; the axostyle and peripheral rod originating with the recurrent flagellum and one other flagellum from one blepharoplast; and the pair of flagella originating from the other blepharoplast. Holl. Prot. × 9,600.

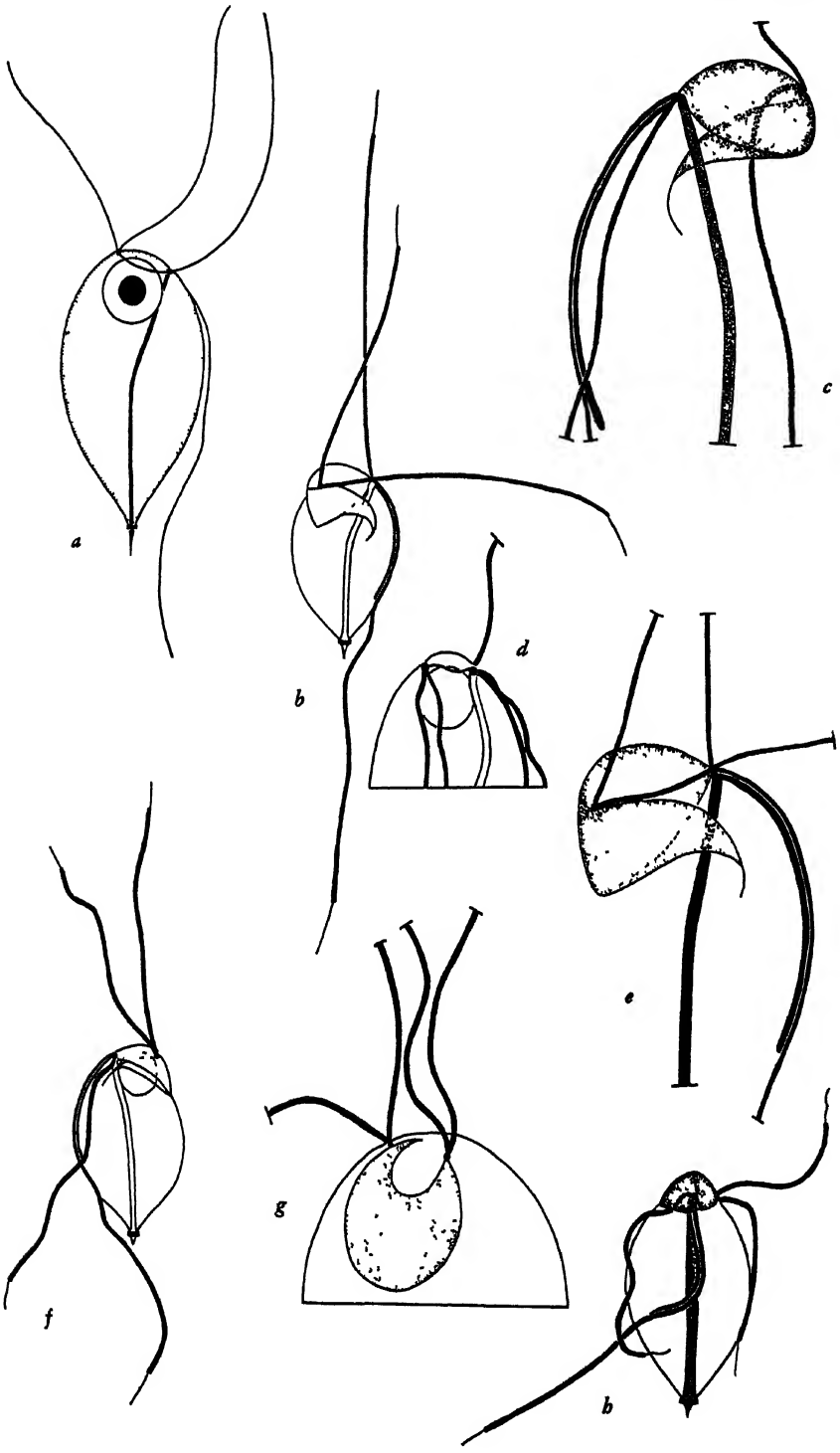
*d.* Anterior end of body showing the two blepharoplasts, the filament connecting the blepharoplasts, the origin of the mastigont structures, and an outline of the nucleus. Holl. Prot. × 3,600.

*e.* Diagram similar to *c* from the opposite aspect. Holl. Prot. × 9,600.

*f.* Diagram similar to *b*, from the opposite aspect. An outline of the nucleus is shown. Holl. Prot. × 3,600.

*g.* View of the pelta from its broad middle part, and origin of the two pairs of flagella. Holl. Prot. × 9,600.

*h.* View of the body with the recurrent flagellum and peripheral rod above, rod and flagellum curved. The pelta appears as a cap-like structure at the anterior end. Holl. Prot. × 3,600.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## PLATE 32

*Hexamitus pulcher* Becker from *Citellus t. tridecemlineatus*

*a*. Specimen represented from the side where one nucleus is above the other, showing the peripheral rods at the ends of which the anterior flagella become free, a caplike structure at the apex, and the caudal flagella that become free lateral to the terminal part of the median axial structure. Holl. Prot. × 4,330.

*b*. Similar figure, showing the stained filaments that constitute the intracytoplasmic posterior flagella, extending along or in the median axial structure. Holl. Heid. × 4,330.

*c*. Posterior part of the body showing the sharp projecting terminus of the median axial structure and the origin of the free caudal flagella. Holl. Prot. × 4,330.

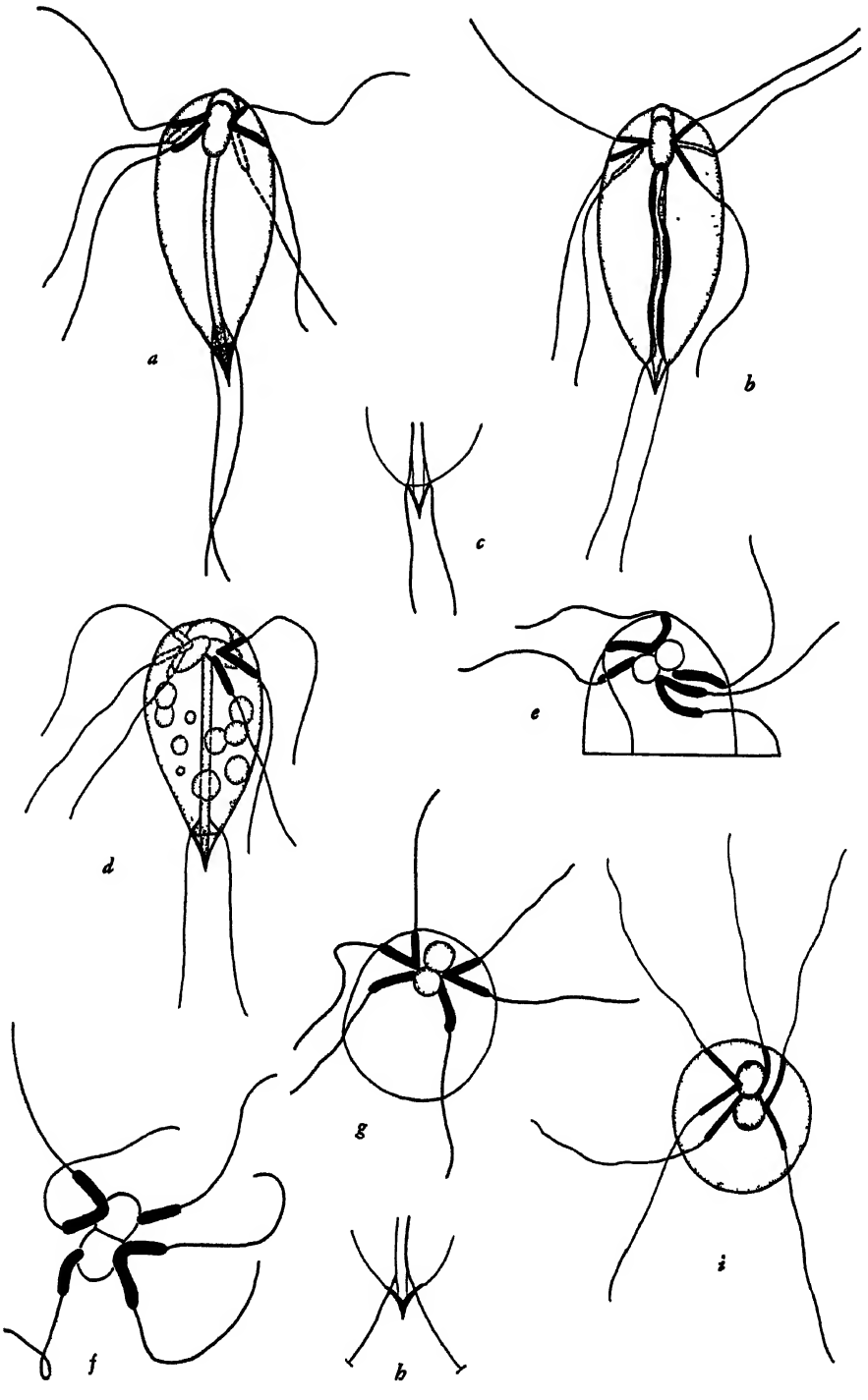
*d*. View from the side where the two nuclei are in one horizontal plane, showing the anterior caplike membrane, the rods related to the anterior flagella, the median axial structure, the caudal flagella, and spherules in the cytoplasm. Holl. Prot. × 4,330.

*e*, *f*. Apical view, showing the two nuclei and the alternating rods and V's at the origin of the anterior flagella. Holl. Prot. × 4,330.

*g*. Apical view, showing rods and V's paired instead of alternating. This is an unusual arrangement. Holl. Prot. × 4,330.

*h*. Diagram of the posterior axial structure and caudal flagella. Holl. Prot. × 4,330.

*i*. Apical view of the anterior rods shown in an iron-haematoxylin preparation. S. Heid. × 4,330.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## PLATE 33

*a–f. Hexamitus teres* n. sp. from *Citellus t. tridecemlineatus.*

*a, c.* Diagrams from the side where the two nuclei are in one horizontal plane. The rods of three anterior flagella meet in a group of three on one side; the others are not visible. The posteriorly flaring axial structures are somewhat twisted, and the caudal flagella emerge at the funnels. *a,* Holl. Heid.; *c,* Holl. Prot. × 4,330.

*b.* Diagrams of the two nuclei, showing the spherical form and the central endosome. Holl. Heid. × 8,660.

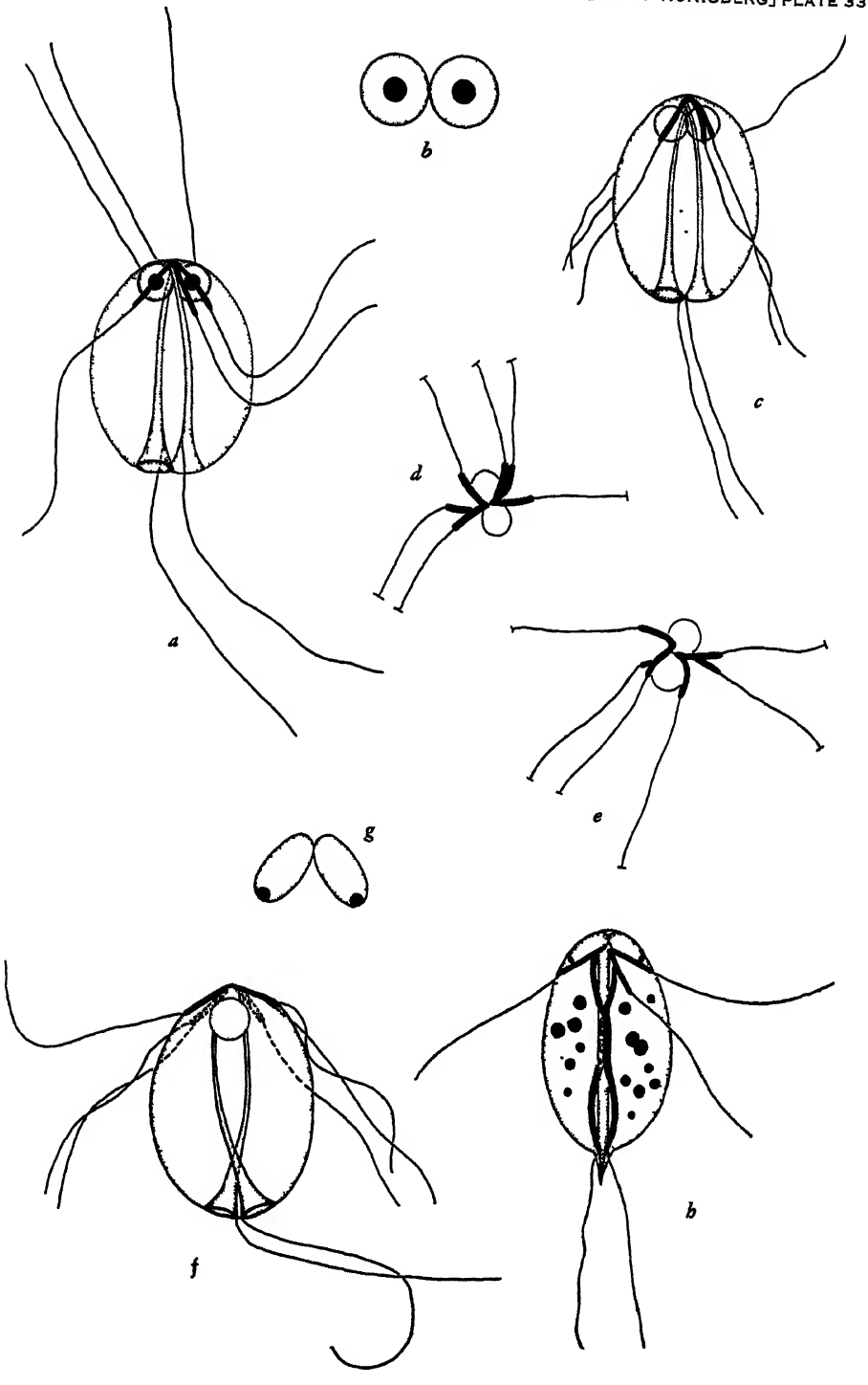
*d, e.* Views from the anterior end, showing the two nuclei in outline and the two groups of anterior rods, from which the anterior flagella continue free. Holl. Prot. × 4,330.

*f.* Diagram from the side where the two nuclei are one above the other, showing the position of the two groups of rods at the periphery of the body, the normal position of all flagella, and the crossing of the separated longitudinal structures in their posterior parts. Holl. Prot. × 4,330.

*g, h. Hexamitus pulcher.*

*g.* From *Citellus t. tridecemlineatus.* Diagram of the two elongate nuclei with posterior endosomes. Holl. Heid. × 8,660.

*h.* From *Citellus b. beldingi.* Stained spherules in the cytoplasm; stained filaments, somewhat spiralled, along the median axial structure, continuing in the caudal flagella. B. Hematein. × 4,300.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## PLATE 34

*Hexamastix citelli* (Wenrich) from *Citellus lateralis chrysodeirus*. Holl. Prot. × 4,330.

*a*. General diagram showing the five anterior flagella with knobs at the ends, the acronemic type of recurrent flagellum, the upper part of the curved pelta, the nucleus with an endosome, the parabasal body beneath the nucleus, and the axostyle.

*b*. The anterior part of the axostyle, not broadened from this aspect, and apparently continued in the impregnated, curved, anterior pelta; parabasal body applied to the nucleus.

*c*. Pelta represented at the apex of the body, elongate nucleus with endosome.

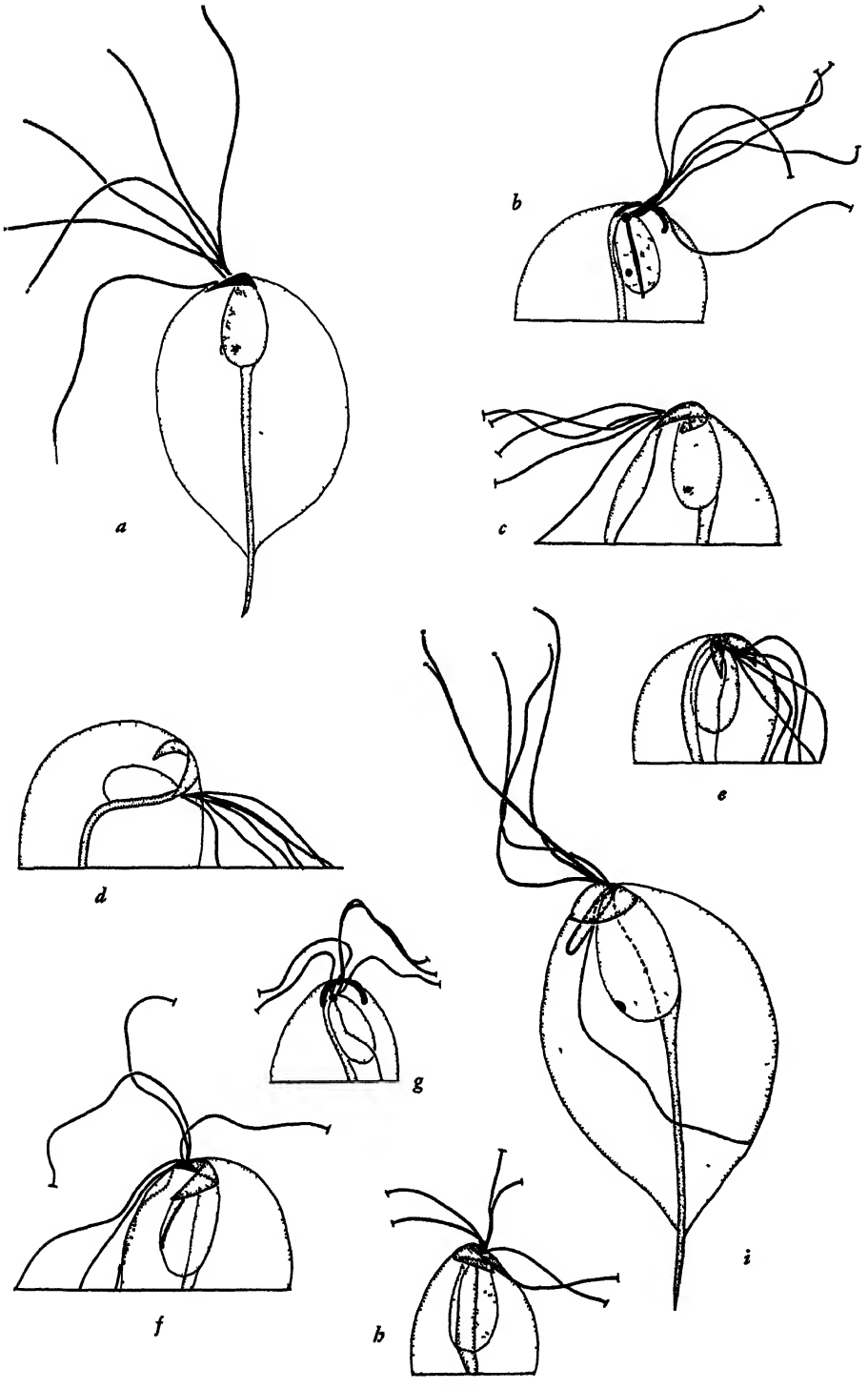
*d*, *e*. The anterior part of the axostyle in its topographic relation to the pelta, and the origin of the flagella.

*f*. Another view of the curved pelta and the origin of the flagella; parabasal filament under nucleus.

*g*. Diagram similar to *b*, parabasal body not shown.

*h*. Showing the moderate broadening of the anterior part of the axostyle seen from this aspect, and the curved pelta.

*i*. Diagram of the whole flagellate, showing the parabasal body, the caplike appearance of the pelta from this aspect, and the characteristic spatulate form of the capitulum of the axostyle.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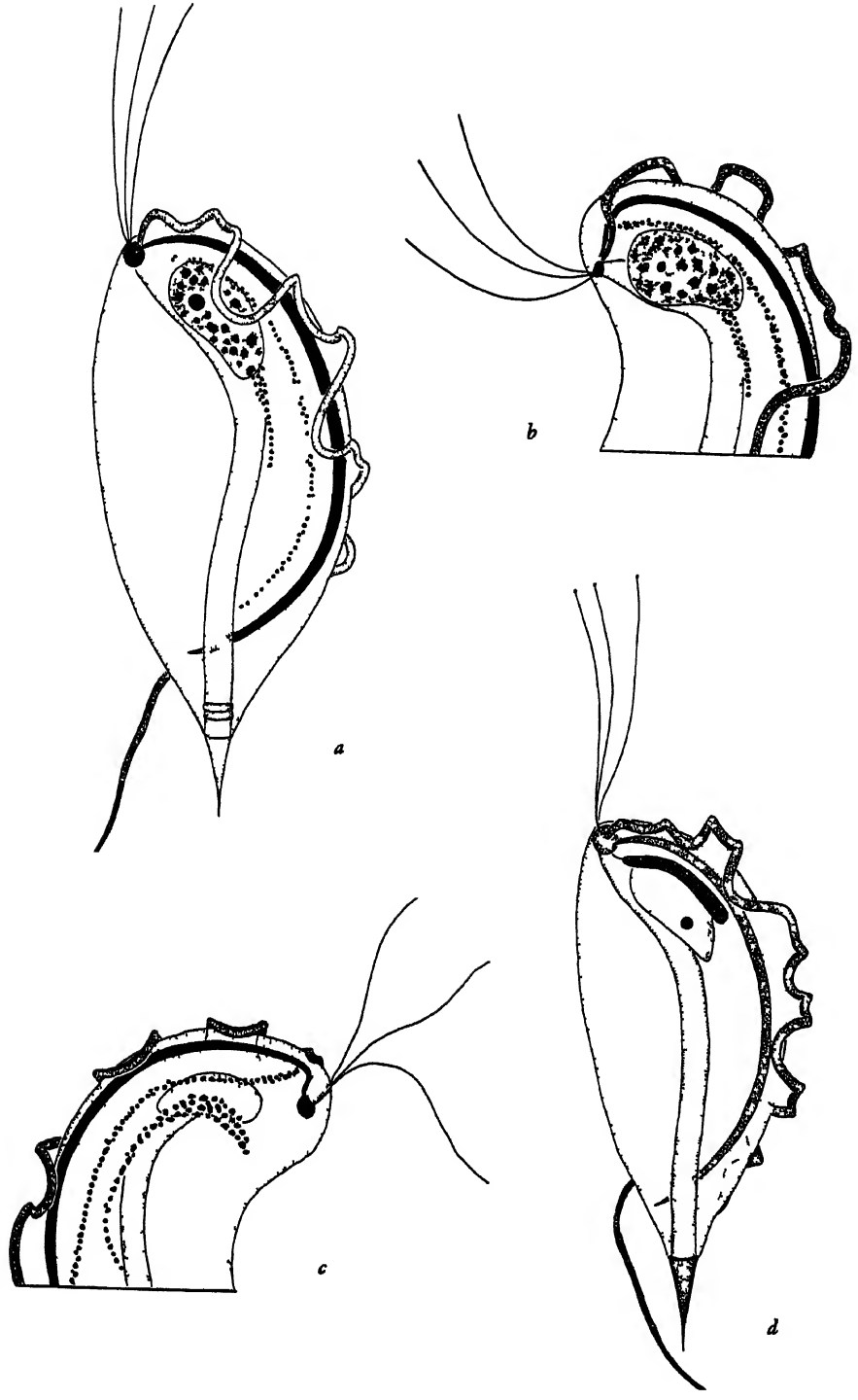
## PLATE 35

*Trichomonas muris* from *Cricetus lateralis chrysoderus*

*a* General diagram, showing the relatively large spherical blepharoplast, the three short, slender anterior flagella, the characteristic shape and structure of the nucleus, the ribbon formed recurrent flagellum along the undulating membrane, the rings around the posterior part of the stout trunk of the axostyle, and the rows of paraxostylar and parabasal granules B Hematein ×4 330

*b, c* Anterior part of the body from opposite aspects, showing the blepharoplast, the anterior flagella, the ribbon formed marginal flagellum of the undulating membrane, and the rows of cytoplasmic granules B Hematein ×4,330

*d* General diagram showing the parabasal body, the relationship of the anterior part of the axostyle to the nucleus, the impregnated granules at the ends of the anterior flagella, and the surface impregnated projecting end of the axostyle Holl Prot ×4 800


a
b
c
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## PLATE 36

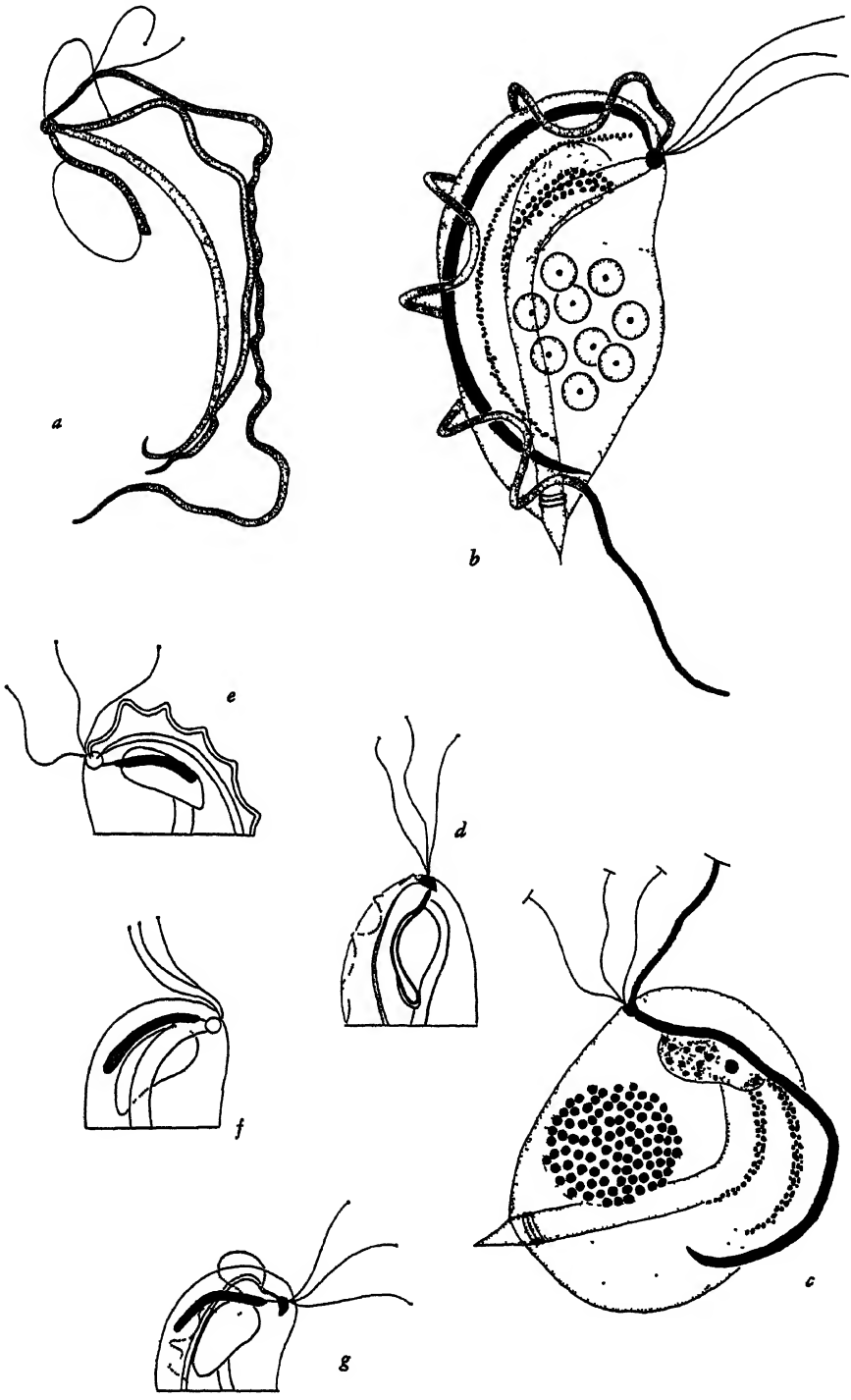
*Trichomonas muris* (Grassi) from *Cricetus lateralis chrysoderus*

*a*. Diagram showing the filament at the border of the undulating membrane, the recurrent flagellum which has separated from the membrane, the costa, the parabasal body applied to the dorsal surface of the nucleus, the blepharoplast, and the three slender anterior flagella. Holl. Prot. × 3,600.

*b*. Flagellate parasitized by a microorganism, "*Sphaerita trichomonadis*." The specimen shows well the groups of para-axostylar granules in their relation to the anterior part of the axostyle, the absence of expansion in the capitulum, the row of paracostal granules, and other structural features. S. Heid. × 4,330.

*c*. Flagellate parasitized by a different microorganism from that of *b*. It appears as a globular group of granules. S. Heid. × 4,330.

*d–g*. Diagrams showing the form and topographic relationships of the parabasal body. Holl. Prot. × 3,600.


a
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# RESPIRATORY METABOLISM OF CERTAIN REPTILES AND AMPHIBIA

BY

S. F. COOK

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# RESPIRATORY METABOLISM OF CERTAIN REPTILES AND AMPHIBIA

BY

S. F. COOK

(Contribution from the Division of Physiology, Medical School, University of California)

## INTRODUCTION

IN THE EARLY spring of 1941 the author, in collaboration with Dr. Robert E. Smith, then at the University of California, initiated a series of studies designed to explore the field of metabolism in small terrestrial cold-blooded vertebrates. The great work of Benedict (1932) had brought to light many interesting facts concerning the large reptiles; Wells (1935) and Sumner and Doudoroff (1938) had opened up the field of respiratory metabolism in fishes; and many investigators were active in the realm of mammalian metabolism. Knowledge of the small reptiles and the amphibia was needed to fill the gap in the vertebrate series.

This investigation was begun and was progressing favorably when the Second World War intervened and forced complete discontinuance. Since the close of the war there has been no opportunity to make a new beginning. Hence it seems appropriate to record the results obtained up to December of 1941, fully recognizing that the data are incomplete and that any conclusions drawn from them can be only tentative.

## MATERIAL AND METHODS

The experimental material included two salamanders, *Batrachoseps attenuatus* and *Aneides lugubris*, both collected during the winter months in Berkeley, and several lizards: *Xantusia vigilis*, *Dipsosaurus dorsalis*, *Uma notata*, *Coleonyx variegatus*, and *Cnemidophorus tessellatus*. The lizards were all taken during April in the southern deserts between Walker Pass and Palm Springs. The salamanders were kept on damp earth in jars in the laboratory and were occasionally fed small insects such as termites. No attempt was made, however, to keep specimens for long periods since the supply could be easily renewed at any time. The lizards were maintained in large cages or glass aquaria on leaf litter or sand. The room was warm and sunny. The feed consisted of meal worms, termites, and dandelion blossoms. The animals remained in good condition, without appreciable loss of weight, well into the subsequent early winter.

Metabolism was measured by a simple modification of the Warburg manometric method of determining the oxygen consumption and carbon dioxide production. The horizontal limb from the manometer which connects normally with the respiration vessel was replaced by a very heavy, thick-walled rubber tube which in turn was attached to a nipple set into the middle of an ordinary metal screw cap. The latter could then be screwed down on a glass

mason jar of any desired volume. The connection was sealed with a rubber ring. Careful preliminary trials showed no appreciable air leak even when the jar was set into a water bath and the temperature varied between wide limits. An alkali inset, placed out of reach of the animal, eliminated carbon dioxide and permitted the direct measurement of oxygen consumption. Parallel determinations with and without alkali measured the carbon dioxide production.

It was of course impossible to secure a basal metabolic rate in the human and clinical sense. As an alternative it was necessary to utilize the concept of *standard* metabolism.[1] The latter envisages a condition wherein the animal shows physical activity but the degree or level of activity is not excessive and is reproducible. With reptiles and amphibia maintained undisturbed in a jar, in a moderate or dim light, with no mechanical or auditory stimuli, optimum conditions are obtained. From time to time there will be some spontaneous activity, particularly at higher temperatures, but this may be very adequately compensated by taking readings over a long period so as to secure a good average and by repeating the determinations on the same or similar animals until the mean value has real statistical significance.

The results were expressed uniformly as cubic millimeters of oxygen consumed per animal per hour or per gram weight per hour, depending on which was more appropriate for a particular purpose.

## INFLUENCE OF BODY SIZE

According to classical theory, in the mammal the metabolic rate is a function not of the weight or mass of the animal but of its surface area. This hypothesis is predicated upon the thesis that the heat production is dependent on the mass, whereas the heat loss is determined by the radiating surface. As the animal increases in size the surface increases as the square of the linear dimension and the volume or mass as the cube. Hence, following the surface-volume ratio, the metabolism should be proportional to the two-thirds power of the weight. Expressed as an equation, if $w$ equals weight or mass and $h$ equals heat production or oxygen consumption:

$$h = kw^{\frac{2}{3}}, \quad \text{or more conveniently}$$

$$\log h = \log k + \tfrac{2}{3} \log w.$$

If the data are plotted logarithmically, therefore, the figure should be a straight line with the exponent $\frac{2}{3}$ denoting the slope of the line.

The real existence of this precise relationship has been questioned in recent years with regard to mammals. Much evidence has been adduced to show that in certain members of this class the slope or exponent is not actually two-thirds but nearer three-quarters. Regardless of this controversy, however, it has long been recognized that the lower vertebrates, not being homoiothermic, must

[1] Many investigators have attempted to place such animals under anaesthesia. This procedure, however, involving the use of any known narcotics, leads to as many difficulties as does the attempt to reach a standard rather than a basal condition. Consequently no narcosis was employed in these experiments.

conform to a different set of principles. Indeed the interrelation between mass and surface is still far from clarified. It had been the intention in this investigation to examine carefully the weight, surface, and heat production of a salamander, *Triturus torosus*, but the work was stopped before unequivocal results were secured.

It was possible, however, to determine the slopes of the lines for several other species. The measurements of oxygen consumption were all made at 20° C. The numbers in parentheses indicate the number of separate determinations.

| *Animal* | *Numerical value of slope* |
|---|---|
| Xantusia | 0.892 (24) |
| Dipsosaurus | 0.930 (48) |
| Cnemidophorus | 0.984 (31) |
| Uma | 0.910 (59) |
| Aneides | 0.715 (83) |

None of these slopes conforms to the two-thirds rule. Furthermore, the four lizards all show quite high values (0.89 to 0.98), whereas the salamander is very much lower. Some preliminary observations on *Triturus* indicated that this salamander also shows a slope in the vicinity of 0.7. Measurements on *Batrachoseps* were made in groups. Within each group the animals were of almost identical weight and hence the mean respiration of a group could be taken as that characteristic of any individual of comparable size. The total number of groups is too small for a very reliable determination of the slope. However, a rough plot gives a visual estimate somewhere between 0.7 and 0.8. The relation between mass and surface therefore appears to be different in lizards from that in salamanders. No explanation can be given at present save the suggestion that the cause may lie in the possibility of respiration through the moist and relatively thin skin of the salamander, whereas the integument of the lizard is dry, thick, and presumably impermeable to oxygen.

## INFLUENCE OF TEMPERATURE

The respiratory gas exchange of all the animals was studied through a range of temperatures extending from 4° C to as high as 36° C. A few tests were made in a bath of ice water with a temperature of 0.3° C. When exposed to extreme cold both salamanders and lizards went into a state which might be described as cold rigor. They became motionless and in some cases so stiff that they could not be bent with the hands. Nevertheless, recovery under warm conditions was always very rapid. Meanwhile metabolism continued, although at a very slow rate. As the temperature was raised spontaneous activity uniformly increased, as might be expected, up to an optimum. Thereafter the animals became sluggish, then motionless, and finally, if the temperature approached the lethal limit, they died. It is noteworthy that the lizards of all five genera endured a temperature of 36° C without serious aftereffects but the salamanders of both genera reached their limit at 28° C and were killed at a temperature of 32° C.

The data relevant to the effect of temperature on gas exchange may be presented first in tabular form. In the accompanying table are given the values for oxygen consumption in terms of cubic millimeters of oxygen consumed per

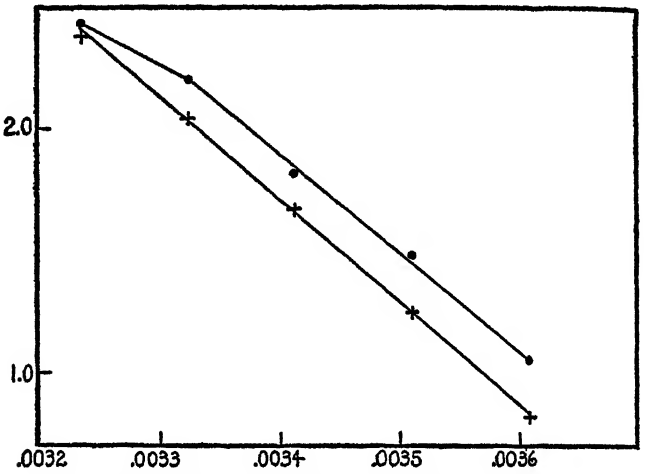


Fig. 1. Temperature curves. The ordinate is the logarithm of the oxygen consumption per gram per hour and the abscissa the reciprocal of the absolute temperature. *Xantusia* is represented by dots and *Dipsosaurus* by crosses.

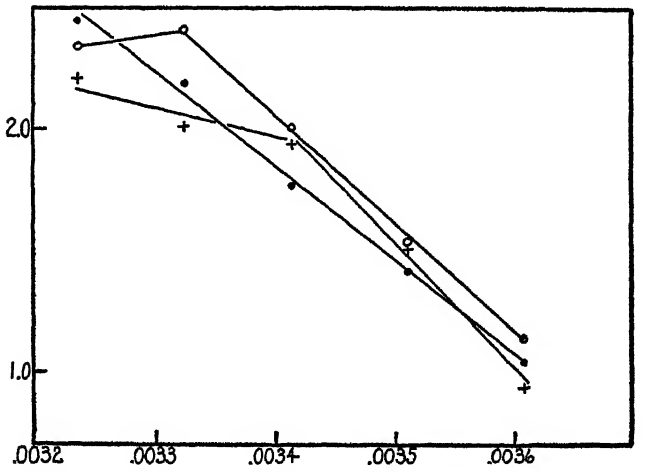


Fig. 2. Temperature curves. The ordinates are the same as in figure 1. *Coleonyx* is represented by dots, *Uma* by crosses and *Cnemidophorus* by circles.

gram per hour for the entire range of temperatures employed. Each value is the mean of at least six, and usually many more determinations.

The differences between the animals of the various genera are depicted in a more striking manner when the data are plotted graphically. Figures 1 to 3 show the temperature curves when the logarithm of the oxygen consumption

is plotted against the reciprocal of the absolute temperature. As is frequent in such graphs of temperature the relationship is linear. Moreover for three of the genera, sharp breaks in the graphs show a discontinuity in the relationship at certain critical temperatures.

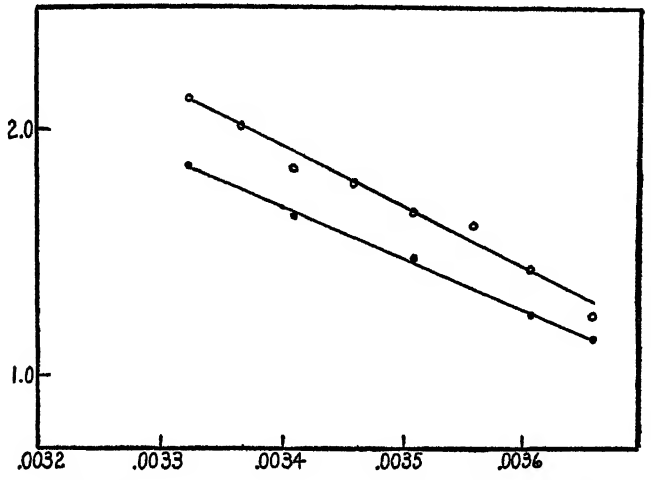


Fig. 3. Temperature curves. The ordinates are the same as in figure 1. *Aneides* is represented by dots and *Batrachoseps* by circles.

| Animal | 0.3° | 4° | 8° | 12° | 16° | 20° | 24° | 28° | 36° |
|---|---|---|---|---|---|---|---|---|---|
| Xantusia | .... | 11.1 | .... | 29.8 | .... | 65.2 | ..... | 160.5 | 272.0 |
| Dipsosaurus | .... | 6.6 | .... | 17.4 | .... | 47.3 | ..... | 109.5 | 242.0 |
| Coleonyx | .... | 10.8 | .... | 25.7 | .... | 57.2 | ..... | 152.0 | 278.0 |
| Uma | .... | 8.5 | .... | 32.2 | .... | 85.2 | ..... | 104.5 | 162.0 |
| Cnemidophorus | .... | 14.0 | .... | 34.7 | .... | 102.5 | ..... | 257.0 | 219.0 |
| Aneides | 14.1 | 17.5 | .... | 29.8 | .... | 44.7 | ..... | 70.0 | ..... |
| Batrachoseps | 17.4 | 27.5 | 40.7 | 46.3 | 61.0 | 69.2 | 103.5 | 132.0 | ..... |

The customary method for expressing the quantitative value of the temperature-respiration relationship is by means of the slope of the lines. The latter is calculated, in each instance, with the use of the equation

$$ln\ (\text{rate at } T_2) - ln\ (\text{rate at } T_1) = \frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

where *ln* is the natural logarithm, "rate" is the oxygen consumption per gram per hour, $T_1$ is a selected lower temperature and $T_2$ a selected higher temperature. The constant $\mu$ is the so-called critical thermal increment or the temperature "characteristic." According to the theory originally developed by Arrhenius and widely applied by Crozier to biological processes, it represents the heat of activation of the reaction which controls the process under con-

sideration. The theoretical significance of $\mu$ has been the subject of much controversy and need not concern us here. However it is highly useful as a purely empirical expression for the intensity of the temperature effect. The following table gives the characteristics for the graphs in figures 1 to 3:

| *Animal* | *Value of* $\mu$ |
|---|---|
| Xantusia (4°–28°) | 18,770 |
| Xantusia (28°–36°) | 12,000 |
| Dipsosaurus (4°–36°) | 16,730 |
| Coleonyx (4°–36°) | 17,820 |
| Uma (4°–20°) | 23,780 |
| Uma (20°–36°) | 5,500 |
| Cnemidophorus (4°–28°) | 20,610 |
| Aneides (0.3°–28°) | 11,000 |
| Batrachoseps (0.3°–28°) | 11,250 |

All five genera of lizards, in the lower temperature range, yield characteristics between 16,000 and 24,000. Of these the highest is shown by *Uma* and the next by *Cnemidophorus*. The former is very active and inhabits sandy spots in the Colorado desert which may become exceedingly hot in the summer. The latter is likewise found in very hot habitats and is unusually active physically. Of the other three genera two (*Xantusia* and *Coleonyx*) are nocturnal and the third (*Dipsosaurus*) is relatively sluggish in its behavior. Now *Uma* and *Cnemidophorus* show clear breaks in their temperature graphs, the former in the vicinity of 20° and the latter near 28°. *Cnemidophorus* indeed actually has a negative temperature characteristic (if such a thing is possible) between 28° and 36°. But *Dipsosaurus* and *Coleonyx* give evidence of no such effect. *Xantusia* appears to lie in an intermediate position, with a slight to moderate effect above 28°. One cannot maintain that the data here reported demonstrate unequivocally a direct association between metabolic organization and habitat or neuromuscular constitution, but the problem here posed merits further experimental exploration.

The values of $\mu$ characteristic of the two genera of salamanders (11,000 and 11,250), while constant throughout the entire temperature range studied, are notably below those found for the lizards. This may indicate an inherent genetic or phylogenetic difference or it may be associated with activity level or type of habitat. Speculation, however, serves no useful purpose. In order for comparisons and correlations to assume great theoretical significance, it would be necessary to examine the temperature curves of a large number of species selected with reference to both phylogenetic and ecological factors.

## RESPIRATORY QUOTIENT

With some of the animals the carbon dioxide production as well as the oxygen consumption was measured. This made possible the calculation of the respiratory quotients. Unfortunately because of the rather high variability of the data, not enough measurements were made to be absolutely certain of the results in detail. Nevertheless, the broad picture is reasonably clear, as demonstrated by the following table. In this table it has not been feasible to break

the data down according to individual genera, hence the results for all the lizards collectively are consolidated and contrasted with those for the salamanders.

The low value of these quotients is remarkable. The R.Q. never exceeds .764 (although some of the individual determinations were higher). The immediate conclusion would be that these reptiles and amphibia were burning fat predominantly (even though at moderate or high temperatures they showed considerable muscular activity). However, before final acceptance of such a conclusion, confirmation of these experiments would be necessary.

| Animal | Mean R.Q. at | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 4° | 8° | 12° | 16° | 20° | 24° | 28° | 32° | 36° |
| Salamanders | .432 | .467 | .629 | .613 | .715 | .760 | .686 | .704 | .... |
| Lizards | .525 | .... | .739 | .... | .764 | .... | .751 | .... | .702 |

The most singular feature of these data, and one which was observed repeatedly, is the very low values obtained with both lizards and salamanders at temperatures ranging from 4° to 12°. A quotient below .6 can scarcely be ascribed to the oxidation of any commonly occurring foodstuff and some other process must be making itself manifest. One possibility would be a massive retention of carbon dioxide in the blood or tissues. However, there appears to be no change in the respiratory quotient at the low temperature throughout several hours of exposure, and when the animal is restored to a higher temperature there is no tendency for the R.Q. to reach unusually high levels as might occur if stored carbon dioxide were rapidly released. The observation must therefore be recorded without further comment.

## INTRINSIC OXYGEN CONSUMPTION

In view of the intergeneric differences brought to light by study of the temperature effect, it appeared possible that there could also be variations in what might be called the intrinsic metabolic rate. By the latter is meant the respiratory rate of a unit weight of tissue at a constant temperature. To obtain such a value the effect of body size must be excluded. This can be done with the data at hand since we know the slope of the line, that is, the numerical constant expressing the relationship between body weight and oxygen consumption. If we let $r$ represent the respiratory rate in terms of oxygen consumption per hour and $w$ represent the weight in grams, then the general equation may be expressed

$$\log r = \log k + x \log w$$

where $k$ is a proportionality constant and $x$ is the exponent of the weight. Knowing $x$ for a particular type of animal and selecting an arbitrary weight, say one gram, we can eliminate the influence of size by substituting the appropriate values in the following equation:

$$\log r_1 = \log r_2 + \log w_1 - x \log w_2 .$$

Here $x$ is the exponent or slope of the line, $w_2$ the weight in grams of the animal for each separate measurement, $w_1$ the desired weight (in this case one gram), $r_2$ the observed respiratory rate expressed as cubic millimeters of oxygen consumed by the whole animal per hour, and $r_1$ the calculated rate of oxygen consumption per gram.

The necessary calculations were made for five of the seven genera here reported. The number of experimental measurements made with *Coleonyx* at 20° C, the temperature selected, were too few for adequate statistical significance. The representatives of *Batrachoseps* used were all quite close to one gram in weight (the average was 0.931 gram) and hence no good respiration-weight graph could be constructed. However, a close approximation is possible since our reference weight is one gram. Using simple proportion involves a slight error but the result will be of the correct order of magnitude. We find therefore the following intrinsic respiratory rates for six genera and their combinations:

| Animal | Intrinsic oxygen consumption per gram per hour at 20° C | | |
|---|---|---|---|
| | cu. mm. $O_2$ | Standard deviation | Standard error |
| Xantusia | 136.6 | ±39.7 | ± 8.5 |
| Dipsosaurus | 51.5 | ±27.0 | ± 3.9 |
| Cnemidophorus | 115.1 | ±83.3 | ±15.2 |
| Uma | 197.4 | ±77.4 | ±10.2 |
| Aneides | 387.0 | ± 1.2 | ± 0.1 |
| Batrachoseps | 75.7 | ..... | ..... |

| Combinations | Mean difference | Critical ratio of the means |
|---|---|---|
| Uma-Xantusia | 60.8 | 4.60 |
| Uma-Cnemidophorus | 82.3 | 4.60 |
| Uma-Dipsosaurus | 145.9 | 13.76 |
| Xantusia-Cnemidophorus | 21.5 | 1.24 |
| Xantusia-Dipsosaurus | 85.1 | 9.15 |
| Cnemidophorus-Dipsosaurus | 63.6 | 4.05 |
| Aneides-Uma | 189.6 | 152.00 |

From the above tables it is evident that the different genera of cold-blooded animals differ widely in metabolic rate, that the different rates are definitely not referable to divergence in body size, and that they possess high statistical significance. The gaps are too great to be accounted for by fluctuations in muscular activity or by variations in nutritional state under the experimental conditions employed. They must therefore be regarded as inherent in the species itself, and hence of a fundamental character. Whether the explanation of this phenomenon is to be sought in the structure of the cardiovascular system or in the cell metabolism of these animals, or whether it is referable to genetic or ecological factors can be determined only by further intensive investigation.

## SUMMARY

1. By a modified manometric technique the respiratory metabolism was measured in five species of lizards (*Xantusia vigilis, Dipsosaurus dorsalis, Uma notata, Cnemidophorus tessellatus,* and *Coleonyx variegatus*) and in two species of salamanders (*Aneides lugubris* and *Batrachoseps attenuatus*).

2. When for each species the logarithm of the oxygen consumption per animal per hour is plotted against the logarithm of the weight, straight lines are obtained having slopes with values near 0.9 for lizards and 0.7 for *Aneides.*

3. The temperature characteristics, calculated by means of the Arrhenius formula, lie between 18,000 and 24,000 for lizards and near 11,000 for salamanders, all at moderate and low temperatures. At higher temperatures (up to 36° C) *Xantusia, Uma,* and *Cnemidophorus* show much lower characteristics—perhaps associated with the mode of life of these animals.

4. The respiratory quotients are all in the range of .7 except that at temperatures of 12° C or less the quotients may be even lower.

5. If the effect of body size is excluded, the intrinsic metabolic rate of the seven species, based upon one gram of tissue, differs widely. It is possible that we are dealing here with inherent specific or generic differences.

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# OBSERVATIONS ON FLAGELLUM STRUCTURE IN FLAGELLATA

BY

DOROTHY R. PITELKA

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# OBSERVATIONS ON FLAGELLUM STRUCTURE IN FLAGELLATA

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DOROTHY R. PITELKA

## INTRODUCTION

UNTIL THE advent of the electron microscope the protistan flagellum was generally considered (see for example Calkins, 1933; Hyman, 1940; Kudo, 1946), on the basis of light microscope studies, to consist of two distinct elements: a central or eccentric fiber, the axoneme, and an enveloping cytoplasmic sheath. Opinion differed as to which of these elements was responsible for contractility. The existence of subfibrils within the axoneme had occasionally been reported (e.g., Korschikow, 1923). In addition to these fundamental structures, one or more rows of numerous, fine filaments extending laterally or radially from the flagellum were first described by Loeffler (1889) and later by numerous authors, but their existence as real entities gained only partial acceptance. Many flagella were seen to bear a single terminal fine filament, which was generally supposed to be a free continuation of the axoneme. The presence on the flagellum of either lateral or terminal filaments, or in some cases both, was observed by authors who were able to demonstrate them to be a remarkably constant character within the larger systematic categories, and the suggestion was made that flagellum structure might have taxonomic significance (Vlk, 1938).

Recent studies employing the electron microscope, with its relatively tremendous resolving power, have considerably refined our knowledge of flagellar structure, although much remains to be learned. Many protistan flagella, as well as cilia and sperm tails, are seen to consist, at least in part, of several longitudinal fibrils (e.g., Schmitt, review, 1944; Jakus and Hall, 1946). In some cases no other structure is revealed; in others a sheath is found, containing or consisting of a closely wrapped helical fibril (Schmitt, Hall and Jakus, 1943; Brown, 1945). Furthermore, the lateral filaments, or mastigonemes, show clearly on some, but not on all, electron micrographs of certain species (Brown, 1945); their significance and point of origin are not understood.

The almost invariable demonstration of longitudinal fibrillar organization in the relatively few flagella thus far electronically examined obviously makes it desirable to study numbers of additional species to discover whether such organization is actually universal in these contractile organelles. The need for further inquiry into the occurrence and structure of sheath and mastigonemes is patent. Furthermore, the possibility that certain aspects of flagellar structure are constant within, and characteristic of, groups of related flagellates needs to be probed by careful study of several series of closely related species. This paper presents the results of a comprehensive study of six species of the

order Euglenida, employing specimens stained for light microscope examination, living organisms seen in dark-field, and electron micrographs of dried specimens. All of the generally recognized families except the Colaciidae are represented.

Deflandre (1934) has suggested a useful set of terms for the designation of flagellar types on the basis of the presence and position of appendages. Although evidence to be presented here indicates that lateral and terminal filaments may be products of degeneration, yet they are distinctive and characteristic enough to warrant use of such terms. Deflandre's terminology will be employed as follows: (1) acronematic flagellum, bearing a single terminal filament; (2) stichonematic flagellum, bearing a single row of lateral mastigonemes; (3) pantonematic flagellum, with two or more rows of mastigonemes, the question of bilateral versus multilateral origin of mastigonemes in these cases being still unsettled; (4) pantacronematic flagellum, with a terminal filament plus two or more rows of lateral mastigonemes (a stichacronematic flagellum has never been described); (5) simple flagellum, with no structure of this order, or none described. Fischer's (1894) German term, "Flimmer" for mastigonemes has been employed by Brown (1945), but, although it has priority, I believe that Deflandre's complete terminology is more manageable in English. The use of the term "cilia" for mastigonemes (Foster *et al.*, 1947, and others) seems to me unreasonable, since the word refers to well-defined structures which most obviously are not mastigonemes. Deflandre's use of "mastigoneme" for the terminal filament of acronematic flagella is likewise to be rejected, since there is no evidence to indicate that they represent similar structures.

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## REVIEW OF LITERATURE

A comprehensive survey of the work of previous authors on flagellar structure is presented in the recent paper of Brown (1945). For the purposes of this study, a discussion, chiefly of some papers which have dealt with details of the euglenoid flagellum, as well as of recent electron microscope investigations, is necessary.

Fischer (1894), employing a mordant and stain developed by Loeffler (1889), was the first to note the presence of mastigonemes on the flagellum of a euglenoid, *Euglena viridis*. They were arranged in a single row, appearing on only one side of the flagellum at any point, and distributed from the base to nearly the tip. All seemed to be of the same length and were oriented in the same direction on any one flagellum. He believed the mastigonemes were active and coördinated, possibly serving to direct currents of water toward the reservoir. He also made observations of internal structure and corroborated a previous report by Klebs (1883) of the extreme susceptibility of euglenoid flagella to disintegration following mechanical disturbance. Transfer of a drop of culture to a slide was found by Klebs in many cases to initiate a destructive process characterized by swelling of the tip of the motile flagellum, followed by casting and rapid disintegration with spherule formation. Susceptibility varied with the species, and many flagella tended to roll up after being cast.

Dellinger (1909) subjected flagella of *Euglena sp.* and *Chilomonas paramecium* to mechanical pressure between slide and cover slip and reported that each flagellum separated into four distinct fibrils of equal length. A number of species of phytomonads and euglenoids were studied by Korschikow (1923) who reported that in these groups the flagellum consisted of a thin axial thread, surrounded by a contractile protoplasmic sheath, itself differentiated into a contractile substance proper and an outer layer. At the onset of disintegration, the intermediate contractile substance was the first to be destroyed at some point along the flagellum. The outer layer became distended at this point and the axial thread was drawn into the vesicle thus created, forming a coil. Ultimately the blister broke and the axial thread again stretched out. The axial thread, he stated, consisted of a very large number of thin fibrils. In *Peranema trichophorum* the axial thread consisted of three thick fibers which became separated from one another when the organism was killed with gentian violet.

Mainx (1928) described for the flagella of some euglenids a central or eccentric axial thread surrounded by a layer of fluid plasma which on destruction aggregated in drops. Employing Loeffler's technique, he saw unilateral mastigonemes on the flagella of *Euglena viridis* and *Phacus pleuronectes* and published good photographs of the latter. He believed the mastigonemes were probably rigid and inactive, serving to increase the surface of the flagellum. He examined but was unable to demonstrate mastigonemes on the flagella of *Euglena rubida, E. anabaena, E. gracilis* and *Rhabdomonas incurvum* (= *Menoidium incurvum*).

Petersen (1929), also using Loeffler's stain, reported stichonematic flagella from several species of *Euglena*, *Phacus pyrum*, and *Trachelomonas volvocina*, although other species of *Trachelomonas* appeared to have simple flagella. Both he and Mainx suggested that submicroscopic fimbrillae might be present on flagella on which they were not demonstrable by known techniques. Petersen's review pointed out the strikingly uniform appearance of flagellar types within the larger systematic categories. He, like Fischer, believed the mastigonemes were motile.

Deflandre (1934) studied a number of species of euglenoids, as well as other forms, using a nigrosin background stain. He reported stichonematic flagella for species of *Euglena*, *Astasia*, *Phacus*, *Trachelomonas*, *Rhabdomonas* (= *Menoidium* in part), *Lepocinclis* and *Distigma*. From observations on *Euglena gracilis* and *Astasia dangeardii*, which were found to have identical flagella, he reported that the mastigonemes were spaced about 1.0 to 1.5$\mu$ apart along the flagellum, were 3.0 to 3.5$\mu$ long, and of a thickness calculated at about 0.5 to 0.8$\mu$. The angle between mastigoneme and flagellar axis was not usually constant, even on a single flagellum, and he assumed that the filaments were supple and articulated. He did not find mastigonemes on some species of the above genera and believed that here they were too short and thin to be detectable. He also studied *Peranema trichophorum*, *Petalomonas mediocanellata*, *Anisonema acinus* and *Entosiphon sulcatum* without discovering any mastigonemes.

A comprehensive review and discussion of previous work on flagellar appendages was presented by Vlk (1938), who had also studied many additional flagellates. His characterization of flagellar types may be cited here. In the acronematic flagellum, according to him, the basal portion was usually relatively stout, and showed a tendency to stretch. The relative sizes of flagellum proper and the terminal filament varied in different species; the filament might be only a submicroscopic tip or might be as much as three times the length of the base; the filament might be tightly rolled or coiled, or it might be relatively rigid and nearly as thick as the base. Flagella with mastigonemes, he said, varied in the length and spacing of the latter, and in the angle at which mastigonemes were set on the axis, as well as in the arrangement of the mastigonemes in one or more rows. There was no clear evidence to indicate whether those flagella bearing what appeared to be two rows of mastigonemes might not actually be beset on all sides with the fimbrillae.

Up to this time all methods of demonstrating stichonematic or pantonematic flagella had involved the drying in air of specimens to be examined, a fact which lent support to those who argued that the mastigonemes were artifacts (e.g., Korschikow, 1923). Acronematic flagella with relatively heavy whip ends had been seen in life by several investigators. Vlk, however, was able to see pantonematic flagella on living specimens of *Mallomonas acaroides* by dark-field illumination. Two rows of mastigonemes were evident, each mastigoneme being about six times as long as the width of the flagellar axis. His observations were corroborated by colleagues at the German University in Prague.

Other species showed mastigonemes in dark-field after light fixation with iodine.

Vlk proceeded to collect all the available data on flagellum types (according to presence and type of appendage on the flagellum), which he tabulated according to taxonomic groupings; the essential points of his tabulation are given here in table 1. Observations on the dinoflagellates were insufficient to warrant any conclusions, but Vlk's studies seemed to support the evidence of Awerinzew (1907) and Entz (1928) for a band-shaped flagellum. The trailing flagellum of some trichomonads is also band-shaped (Kirby, 1944). Subsequent investigations by Vlk (1939), Couch (1941) and Ellison (1945) of zoospores of some fungi have revealed that acronematic, pantonematic and simple flagella, as well as flagella with knobbed ends, are present on various species. In papers by Couch (*op. cit.*), Bessey (1942) and Ellison (*op. cit.*), evidence of flagellar types was brought to bear on general problems of fungus phylogeny.

A greater part of the electron microscope work bearing on the present problem has been concerned with sperm tails. Baylor, Nalbandov and Clark (1943) reported from electron microscope studies of chicken and bull sperm that the axial filament was made up of numerous long fibrils which appeared in unstained specimens to be free at the end of the tail. Harvey and Anderson (1943) found in sperm of *Arbacia punctulata* that the tails were frayed into about 10 strands of uniform thickness, each with a diameter of about 500Å; regularly spaced cross striations were sometimes observed. Electron microscope studies by Schmitt, Hall and Jakus (1943) revealed that squid and many vertebrate sperm tails separated on drying into 9 to 11 fibrils, each 350 to 600Å wide, and extending the length of the tail without branching or anastomosing. In frayed squid tails, all fibers appeared identical and there was no evidence of a differentiated axial bundle or filament. Some unfrayed specimens showed a greater density in the center (their fig. 4a shows quite distinctly two dense lines in the central region) and the authors suggested as a possible explanation that a symmetrical, fairly close packing of the fibrils with salts, etc., adsorbed might cause an appearance of greater density in the center of the bundle. They found that the tail was less likely to fray if salts in the medium (sea water) were not washed out before drying, and offered the suggestion that the hypotonic washing water might rupture a binding membrane or leach out an embedding substance. A sheath composed of a closely wound helical fiber, also 300 to 500Å thick, was noted, especially on mammalian sperm tails.

Schmitt (1944) in a review of the literature reported that subfibrils had been found in all sperm tails thus far examined, the number of fibrils per tail being constant within a range of 9 to 12. These fibrils were revealed by Schmitt's experiments to be protein. Finer subfibrils were seen occasionally.

The paper of Schmitt, Hall and Jakus (1943) was among the first to include electron micrographs of protistan flagella. The authors found that the flagella of *Trichonympha* sometimes were frayed into evenly contoured parallel fibrils 250 to 400Å in width and extending the full length of the flagellum. Cilia of *Frontonia* and other infusoria were likewise frayed. Jakus and Hall (1946)

TABLE 1

SYSTEMATIC SUMMARY OF FLAGELLUM TYPES (AFTER VLK, 1938)
(P = pantonematic. A = acronematic. S = stichonematic. PA = pantacronematic.)

| | | |
|---|---|---|
| **A. Chrysophyta** | | |
| 1. Chrysophyceae | | |
| *Chromulina* sp. | 1P | |
| *Mallomonas acaroides* | 1P[a] | |
| *M. acrocomos* | 1P | |
| *Synura uvella* | 1P | 1A |
| *Uroglena volvox* | 1P | 1A[b] |
| *Dinobryon sertularia* | 1P | 1A[b] |
| *Monas minima* | 1P | 1A[b] |
| *M. sociabilis* | 1P | 1A[b] |
| *M. amoebina* | 1P | 1A[b] |
| 2. Heterokontae | | |
| *Botrydiopsis arhiza* | 1P | 1A |
| *Heterococcus* sp. | 1P | 1A |
| *Tribonema* sp. | 1P | 1A |
| *Botrydium granulatum* | 1P | 1A[b] |
| **B. Chlorophyta** | | |
| 1. Chlorophyceae | | |
| *Spermatozopsis exultans* | | 2A[a] |
| *Haematococcus pluvialis*[c] | | 2A |
| *Carteria* sp. | | 4A |
| *Chlamydomonas fusiformis* | | 2A |
| *C.* sp. | | 2A |
| *C. dorsoventralis* | | 2A |
| *Chlorogonium euchlorum* | | 2A |
| *C. aculeatum* | | 2A |
| *Lobomonas regularis* | | 2A |
| *Diplostauron angulosum* | | 2A |
| *Brachiomonas submarina* | | 2A |
| *Pteromonas angulosa* | | 2A |
| *Polytoma uvella* | | 2A |
| *Dunaliella salina* | | 2A |
| *Chlamydobotrys gracilis* | | 2A |
| *Gonium pectorale* | | 2A |
| *Dictyococcus irregularis* | | 2A |
| *D. minor* | | 2A |
| *Chlorococcum* sp. | | 2A |
| *Ulothrix zonata* | | 2–4A |
| *Draparnaldia acuta* | | 2–4A |
| **C. Euglenophyta** | | |
| 1. *Euglenaceae* | | |
| *Euglena oblonga* | 1S | |
| *E. geniculata* | 1S | |
| *E. pisciformis* | 1S | |
| *E. gracilis* | 1S | |
| *E. viridis* | 1S | |
| *Phacus pleuronectes* | 1S | |
| *P. pyrum* | 1S | |
| *Trachelomonas volvocina* | 1S | |
| *Astasia dangeardii* | 1S | |
| *Menoidium*[d] *incurvum* | 1S | |
| *Menoidum*[d] *longum* | 1S | |
| *Distigma proteus* | 1S (or 2?) | |
| *D. pseudoproteus* | 2S | |
| *D. minima* | 1S | |
| *Urceolus cyclostomus* | 1S | |
| **D. Zoomastigophora** | | |
| 1. Pantostomatinae | | |
| *Mastigamoeba butschlii* | | 1A |
| *Cercobodo crassicauda* | | 2A[a] |
| 2. Protomastiginae | | |
| *Oicomonas socialis* | 1P | |
| *Codonosiga botrytis* | 1PA or 1A | |
| *Salpingoeca* sp. | 1PA | |
| *Dendromonas virgaria* | | 1A[b] 1A[a] |
| *Bodo* sp. | | 2A |
| *B. mutabilis* | | 2A[a] |
| *B. angustus* | | 2A |
| *B. minimus* | | 2A |
| *Spongomonas intestinum* | | 2A[a] |
| 3. Distomatinae | | |
| *Trepomonas steinii* | | 2A[b] 6A |
| *T. rotans* | | 2A[b] 4A |
| *T. agilis* | | 6A[b] 2A[a] |
| *Urophagus rostratus* | | 8A |
| *Hexamitus inflatus* | | 8A[a] |

[a] Seen in living specimens.
[b] Terminal filament reduced.
[c] = *Sphaerella lacustris.*
[d] = *Rhabdomonas.*

found similar structures in the cilia of *Paramecium*, the fibrils numbering about 11. In specimens fixed before drying, the fibrils usually adhered in one bundle. They saw occasional specimens in which the fibrils appeared as two bundles at the unfrayed proximal end of the cilium. No evidence was reported of any structure which would serve to bind the fibrils together in life, with the exception of a poorly defined cross-striation which was noted in a few cases, and which they suggested could possibly be a remnant of a binding structure.

Brown (1945) studied the flagella of *Astasia klebsii, Euglena gracilis, Ochromonas variabilis* and *Chilomonas paramecium* and reported: "Each flagellum is of approximately uniform diameter throughout its entire length. . . . Each flagellum consists of a denser axial core (axoneme) and a less dense sheath surrounding the core. . . . In the flagella of *Euglena* and *Astasia*, the axial core appears to consist of two closely approximated fibers of equal size. . . . The sheath appears to contain or to consist of a coiled fiber which encircles the axial core in the form of a helix . . ." (p. 262). His electron micrographs are the first to show mastigonemes. On the flagella of *Euglena gracilis* and *Astasia klebsii* these occur in a single row and are about 1.5 to 2.0$\mu$ in length; on the flagellum of *Ochromonas variabilis* they are arranged bilaterally or multilaterally, and Brown estimated their dimensions at about 0.5$\mu$ in length and less than 0.01$\mu$ in diameter. The mastigonemes are here seen in all cases to be twisted and disordered, with none of the neat regularity apparent in some light microscope preparations. Brown suggested that this might result from his use of a centrifuge in preparation of his specimens. In some of his plates, the mastigonemes appear to branch and anastomose to form an irregular network along one side of the flagellum. He offered the suggestion "that the lateral filaments might possibly be due to the escape (and subsequent coagulation) of plasm from a lateral series of minute pores" (p. 262). Brown's plate 3, A, which shows what he interpreted as the helical fiber of the sheath, is reproduced here as plate 37, *a*.

Foster, Baylor, Meinkoth and Clark (1947) on the basis of electron microscope studies of unidentified flagellates classified flagella in two groups, the "ciliary," bearing mastigonemes and with an axis which appeared to be a solid rod or hollow tube or possibly densely packed longitudinal fibers; and the fibrous, without mastigonemes and composed of spirally twisted elongate fibrils. Their micrographs include stichonematic and pantonematic flagella. They suggested that the mastigonemes may play some part in movement of the flagellum or may "serve to increase the sensory, absorptive, or secretory surface of the cell" (p. 120).

Saxe (1947) in an unillustrated abstract described electron micrographs which show the axoneme of the flagellum of *Euglena gracilis* to consist probably of numerous longitudinally oriented components. No mastigonemes were demonstrated. The flagella of *Octomitus intestinalis* (= *Hexamitus intestinalis*) appeared to consist of a dense axoneme and less dense sheath. Dinichert, Guyénot, and Zalokar (1947) published electron micrographs of single cilia from the comb plates of the ctenophore, *Pleurobrachia*, which show, in fixed

specimens, a dense axis and less dense sheath. The axis, in specimens not fixed before drying, frayed into several filaments 250 to 300Å in diameter.

A single recent contribution to light microscope studies of flagella is a paper by Owen (1947) comparing the effects of various fixing and staining techniques on these organelles. He concluded that mastigonemes are demonstrable only by dry fixation (in spite of his cognizance of Vlk's work) and that they are therefore fixation artifacts explainable either by Brown's hypothesis of extrusion of plasma through the flagellar membrane, or by the dislodgment of micelles from the sheath, as had been suggested by Barker (1943). Owen found that a modification of Gelei's (1926) osmic-acid-formalin solution was the most satisfactory fixing agent for flagella, which never showed mastigonemes when fixed thus. Loeffler's technique proved the most reliable staining procedure.

## MATERIALS AND METHODS

The organisms studied were from laboratory cultures of *Euglena gracilis*, *Astasia longa*, *Rhabdomonas incurvum*, *Peranema trichophorum*, *Peranema acus* and *Entosiphon sulcatum;* some studies of *Sphaerella lacustris* and *Monas vestita* are included for comparison. *Euglena*, *Astasia*, and *Sphaerella* were grown as pure cultures in bacteria-free media; all others in ordinary pea, wheat, hay, or soil media with at least one other protist and abundant bacteria.

For observations with the light microscope, the stains of Deflandre (1923) and Loeffler (1889; see McClung, 1937) were used most extensively. In addition, fresh dilute gentian violet was used as recommended by Korschikow (1923); other flagellar stains were tried but none gave results superior to these. Deflandre's nigrosin relief stain is useful as a quick method of demonstrating the contours of flagella and the presence or absence of appendages. Loeffler's mordant and stain were found to be the most valuable of the light-microscope techniques employed in study of both internal and external structure of flagella, in spite of widely variable results. Fixation was accomplished usually by allowing a drop of water containing concentrated living organisms to dry on a slide. Rapidity of drying was varied by the use of a vacuum desiccator, by placing the slides about six inches from a 60-watt electric bulb, or by permitting the slides to dry at room temperature and humidity. Some preparations were made, using Loeffler's reagents, but without at any time permitting the specimens to dry in air. To accomplish this, the organisms were killed by adding a drop of 2 per cent osmic acid to 5 cc. of water containing a concentrated suspension of cells. The osmic acid solution was then thoroughly washed out by repeated centrifugation and the mordanting and staining processes were carried on in centrifuge tubes, followed by rapid dehydration in alcohol, clearing in xylol, and mounting in balsam.

Dark-field studies were made of living organisms suspended in distilled water, tap water, or culture medium, and of specimens killed by osmic acid vapor or by Lugol's solution. It was found that in spite of the presence of a water cooling cell in the path of illumination, the heat from the carbon arc lamp necessary for adequate lighting was invariably fatal to the organisms

after five to thirty minutes' observation. This made it possible to watch the gradual cessation of movement and to study the immotile flagellum prior to and during disintegration.

The electron microscope used was an RCA, type B, instrument at the University of California. Specimens were prepared according to the method of Marton (1941), using 1½ per cent Parlodion in amyl acetate to make the supporting film. For most preparations living organisms were suspended in distilled water and then dried in air on the prepared collodion film. This method of fixation would be expected to have a destructive effect on the flagellum and preparations made thus cannot be supposed to represent lifelike structures but rather stages in disintegration. The method was employed because the experience of previous authors with electron microscope work, and of the present author with light microscope studies, showed that internal morphology of the flagellum is frequently more clearly indicated in frayed and partially disintegrated specimens than in chemically fixed, unfrayed ones. In some cases the organisms were first fixed in hot Schaudinn's fluid or in Gelei's (1926) solution of 10 parts of 2 per cent osmic acid and one part formalin, then suspended in distilled water and dried in air. In all cases, cultures were initially concentrated by centrifuging.

The electron microscope was calibrated by its operators, who used a quartz replica of a diffraction grating as a standard of measurement.

As has been pointed out by Brown (1945), rupture of the collodion film in the electron beam is a serious hindrance to electron microscope study of large-bodied organisms such as euglenoids. A further disadvantage in my work was the necessity of using impure cultures of most of the forms employed, where bacteria and debris frequently obscure the structures studied, or make it difficult to determine by visual examination of the microscope's fluorescent viewing screen whether the structure in focus is the desired flagellum. In such cases it was necessary to exclude from consideration all micrographs in which the flagellum was not attached to a recognized euglenoid cell, or in which the flagellum was not identifiable as such by certain characteristics revealed by other techniques.

## ORIGINAL OBSERVATIONS ON FLAGELLUM STRUCTURE

### *Euglena gracilis*

*Stained specimens.*—Almost every appearance attributed to protozoan flagella by previous authors (see Brown, 1945) can be found in Loeffler preparations of this species. Variation, both in intensity of stain and in structures seen, is extreme, even on a single slide. Considerable variations in the speed of drying, or in timing of steps in the staining process, could not be correlated with variations in appearance of the stained flagellum. In general, preparations which were allowed to dry very slowly showed a larger number of frayed or diffuse flagella, but this was not always the case. It seems possible that the clumping of cells in certain areas on the slide, the presence of dissolved salts due to incomplete washing out of the culture medium, or other factors which locally

influence the speed of evaporation of the final film of water during fixation by drying, might cause local variation in the degree and perhaps in the kind of destructive change undergone by the flagellum before fixation is complete; similar factors might influence the character of the stain.

Although many instances have been seen of flagella which are uniformly stained, or which appear vacuolar or beaded, the majority tend to show a differentiation into a dark-stained core and a lighter-stained sheath (see pl. 39, *a*, of *Astasia longa*). In many cases a division of the flagellar core, or axoneme, into longitudinal subfibrils is demonstrable (pl. 37, *b*). Where single or multiple axial fibrils are indicated, the substance of the sheath may appear swollen and diffuse, or may be entirely absent. The frayed axoneme shows two to six fibrils of varying widths.

A phenomenon noted by the majority of previous investigators, which may readily be observed microscopically in dying cells, is the tendency of such cells to cast their flagella; often the flagella subsequently roll up, forming a characteristic tight coil. Of those flagella in my Loeffler preparations which are partly or completely coiled, many are indistinct, but some show a fibrillar knot within the coil, with the sheath substance forming a more or less diffuse globe around them (see pl. 39, *a*, of *Astasia*).

Mastigonemes, when present, are about $3\mu$ in length and are spaced at intervals of approximately 0.5 to $0.7\mu$ unilaterally along the flagellum (pl. 37, *c* and *d*). They may be straight and orderly in arrangement or, more frequently, somewhat tangled and twisted. All the mastigonemes of a single flagellum, however, tend to be oriented in the same direction along the flagellar axis, although the specific angle between mastigoneme and flagellum may vary. Where the flagellum is still attached to the cell, the free ends of the mastigonemes point toward the distal end of the flagellum. If the flagellum is curved, the mastigonemes almost invariably appear on the outside of the curve. When a flagellum shows internal differentiation into axoneme and sheath, the mastigonemes, if present, exhibit the same staining properties as the sheath.

Preparations made by fixation in osmic acid and Loeffler staining without drying show flagella with little internal differentiation. In optical section, the flagellum appears as a dark-stained cylinder, bearing unilateral mastigonemes. As in dried preparations, all mastigonemes on a single flagellum show similar orientation with respect to the flagellar axis, the free ends being directed always toward the distal end of the flagellum where this is identifiable.

A phenomenon seen in these wet-fixed preparations which may be worthy of note, although it was observed with clearness too infrequently to be considered conclusive, is the arrangement of the row of mastigonemes in a loose spiral about the flagellar axis. The spiral corresponds to undulations of the flagellum itself in such a manner that the mastigonemes arise from the outer curvature of each bend in the flagellum, and makes two or three complete turns about the flagellum from base to tip. Vlk (1938) noted that in dried preparations of *Euglena* the row of mastigonemes appeared alternately on opposite sides of the axoneme in an apparently spiral arrangement.

The nigrosin background stain of course yields no information on internal differentiation. Flagella average about 0.6$\mu$ in diameter. Mastigonemes are frequently present, are quite orderly in arrangement, rarely tangled or bent, average about 2.4$\mu$ in length, and are spaced at intervals of about 0.9$\mu$ along the axis of the flagellum. As in Loeffler preparations, mastigonemes appear on the outside of any curvature of the flagellum. They are set at an angle usually of about 35° with the flagellum, although where the flagellum is straight the angle may be less than this; their free ends are directed toward the distal end of the flagellum. Occasionally the diameter of the flagellum decreases sharply near its end, to terminate in a short, slender, usually straight fiber not unlike the terminal filament of some acronematic flagella.

Among my nigrosin preparations a single slide, prepared by the same method as the others, showed large numbers of flagella with mastigonemes differing rather markedly in size from those on the majority of slides. Although the flagella were of about the same diameter as usual, the mastigonemes were very thick, about 3.5$\mu$ in length, and spaced at intervals of about 1.8$\mu$ along the flagellum (pl. 37, *e*). Their arrangement was regular and their orientation normal.

*Dark-field studies.*—The typical reaction of living *E. gracilis* to the rigors of dark-field illumination is a gradual slowing of locomotion, with momentary intervals of complete quiescence during which the flagellum may be observed. Eventually the flagellum is broken off, usually at the point where it enters the neck of the reservoir, and it may continue active contraction for several seconds after being cast. Frequently, before or shortly after the flagellum is cast, a bulbous swelling appears at some point, and may gradually increase in size at the expense of the length of the flagellum until most or all of the flagellum apparently is drawn into the blister, which however still appears optically empty. More often the bleb remains fixed at a relatively small size until a typical granular disintegration of the entire flagellum sets in. Some motility may be retained after bleb formation, in the portion of the flagellum proximal to the bleb.

During moments of inactivity while the flagellum is still attached to the living cell, as well as after the cast flagellum has ceased moving, mastigonemes may be observed, set in an orderly single row along one side of the flagellum. It must be emphasized that they have been seen only on cells which subsequently died or on dead cells or on cast flagella. Their length, spacing, and angle with the flagellar axis are approximately the same as described above for most nigrosin preparations, and again they appear on the outside of any curvature of the flagellum. The flagellum itself appears smooth, optically homogeneous, and about 0.65$\mu$ in diameter (pl. 37, *f*). Mastigonemes may occasionally be seen radiating from the bleb just described.

Mastigonemes were also seen on flagella of an unidentified species of *Euglena* found in a sample of foul brackish water. The appearance of flagellum and mastigonemes was like that described for *E. gracilis*.

Mastigonemes are not always demonstrable on flagella of *E. gracilis*.

Usually they are either present or absent uniformly on all flagella in a single preparation. Since they are visible only under the most favorable conditions of illumination, failure to see them may frequently be due to undetected flaws in the microscopic setup. However, the most exact duplication of optimum optical conditions may on many occasions not reveal any trace of appendages on the living, cast, or fixed flagellum. My observations of these facts have been supported by Profs. J. E. Gullberg and Harold Kirby, both of whom have seen motile stichonematic flagella.

*Electron microscope studies.*—Examination of plate 38, *a* to *e*, shows that, as was found with other methods, the appearance of the dried flagellum varies considerably, although the technique of preparation is the same. It would seem that the flagella shown in plate 38, *a*, *b*, and *e*, have suffered less destruction during the process of drying than have those in plate 38, *c* and *d*. In plate 38, *b*, the flagellum is seen to contain two straight, uniform columns of denser (or thicker) material separated and surrounded by a region of lower density, which in turn is bounded externally by a sharp, thin, limiting membrane. The diameter of the flagellum here is about 0.51$\mu$, while that of each denser column in the interior does not exceed 0.1$\mu$. The two denser central strands might, on the basis of this figure, represent either the walls of a cylinder or separate fibers. The mastigonemes are very numerous and are estimated to be no more than 250Å in diameter; they average 2.5 to 3.0$\mu$ in length. They appear on one side only of the flagellum, but otherwise are without any uniformity in orientation, being considerably tangled and twisted in some areas. There is some suggestion of clumping, for example, in plate 38, *a*, but this for the most part appears to be fortuitous.

Plate 38, *e*, again shows the two darker central strands and the sharp limiting membrane. In several places, two or more mastigonemes are joined to form single fibrils of greater diameter, but no order appears in their arrangement.

Localization of the origin of the mastigonemes is not possible on the basis of these micrographs. In plate 38, *a*, careful examination shows a few places where the base of the mastigoneme appears to cross the lighter region immediately below the limiting membrane. This appearance would result, however, if the flagellum were turned so that the origin of the mastigonemes was not precisely at the edge seen in profile. In the other figures, there is no evidence to indicate that the mastigonemes do not arise from the limiting membrane. On the straight portion of the longer flagellum seen in plate 38, *b*, there is, immediately external to the limiting membrane at the lower edge of the flagellum, a very regular series of short, oblique lines. They are constant in length, spacing, and angle formed with the limiting membrane, and the mastigonemes appear in some regions to extend out from them, as though they constituted the basal portions of these filaments. These oblique striations do not appear to continue internal to the limiting membrane.

Plate 38, *c* and *d*, depict flagella differing considerably from those just discussed. In both figures, a longitudinal fibrillar organization of the flagellum is evident. The frayed lower end of the flagellum in plate 38, *c*, shows seven

fibrils of approximately the same diameter, and one, to the right in the figure, which seems wider and could be composed of two or more similar ones. In neither figure is there any evidence which would explain the double strands previously seen, unless the appearance near the upper extreme of the flagellum in plate 38, *c*, where somewhat more than half of the fibrillar core seems to come together to a blunt, rounded end, be interpreted as a suggestion of a fibrillar bundle.

Surrounding the fibrillar core in the flagella shown in plate 38, *c* and *d*, may be seen an irregular layer of material which presents a frothy appearance. Whether this represents an alveolar or a fibrillar structure is not clear. Along the upper margins of the sections of flagella in plate 38, *d*, this layer is somewhat more compact and displays a certain regularity which bears a faint resemblance to the helical fiber in the sheath shown in plate 37, *a*, and others of Brown's micrographs. Around the frayed portion of the flagellum in plate 38, *c*, this layer is absent, perhaps being represented by dispersed fragments seen to either side. Mastigonemes are entirely absent.

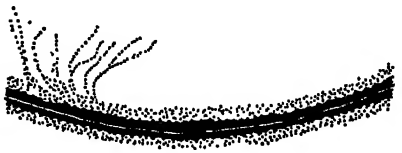

Fig. 1. Part of flagellum of *Euglena gracilis*, drawn from electron micrograph reproduced as plate 38, *g*. See text for discussion.

Plate 38, *g*, represents a portion of a flagellum fixed in osmic-acid-formalin before drying. Plate 38, *f*, apparently shows two such flagella lying partly superimposed. In both figures the core of the flagellum is very dense (as is the case in Brown's osmic-fixed flagella), shows some indication of a double nature in the presence of a slightly lighter streak visible in the center of the core in some areas, and instead of being smooth is sharply and regularly notched all along both margins. Surrounding this is a substance of extremely low density, of uniform thickness, which forms the superficial region of the flagellum, a sharp limiting membrane being absent. Mastigonemes are barely visible along part of the flagellum in plate 38, *g* (fig. 1). They are of the same density as the sheathing layer of the flagellum and appear to arise from and to be continuous with it. Along other regions the margin of this layer is smooth and clear and mastigonemes are quite definitely lacking. Diameter through the core and sheath in plate 38, *g*, is about 0.65$\mu$ or larger than that of the flagellum dried from life.

### *Astasia longa*

*Stained specimens.*—Flagella of *Astasia longa* in Loeffler and nigrosin preparations do not differ in any significant way from those of *Euglena gracilis*. Most of the variations of structure described for the latter species were found on slides of *A. longa*. In my Loeffler preparations, a majority of the flagella were rather broad, flattened, and band-shaped; many of these showed a clear, pale stain internally with darker margins (this heavier deposition of stain along the margins of the flagellum occurred repeatedly in Loeffler preparations of all species studied); others showed one or more axial fibers in a lighter-stained matrix (pl. 39, *a*).

A great many of the cast flagella of *Astasia* were coiled as described above (pl. 39, *a*). Mastigonemes frequently radiate out from these coiled forms and stain as the sheath. On extended flagella, mastigonemes, when present, showed the same dimensions and orientation as in *E. gracilis*.

A large number of *Astasia* flagella were more or less frayed. Plate 39, *b*, shows an example in which a relatively slender, uniformly stained flagellum separates first into two strands of unequal thickness, and, near the end of the flagellum, frays into one heavy and 5 to 6 very fine fibrils separated by a light-staining substance.

In nigrosin preparations, flagella of *Astasia* were occasionally seen from one end of which extended a very fine, wavy thread. This usually appeared on cast flagella but sometimes was seen on the distal end of still attached flagella.

Flagella of an unidentified species of *Astasia* which was found abundantly in an enriched sample of pond scum showed approximately the same structures and variations as those of *Astasia longa*.

*Dark-field studies.*—*Astasia longa* reacts just as does *Euglena gracilis* to dark-field illumination. The former species is somewhat more sensitive to centrifugation than is *E. gracilis*—i.e., the cells are more likely to cast their flagella and ultimately to break or burst if centrifugation is repeated or prolonged; in dark-field preparations of *A. longa*, bleb formation occurs somewhat more rapidly and flagella are more frequently completely rolled up soon after being cast than is the case with *Euglena*. Mastigonemes may be seen occasionally (pl. 39, *c*) but by no means always. The structure and dimensions of the flagellum seen in dark-field are identical in the two species. On a few occasions cast flagella of *Astasia* were seen from one end of which protruded a very thin, somewhat granular, vibrating thread.

*Electron microscope studies.*—Like all other methods employed, electron microscope examination adds to the evidence of essential structural similarity in the flagella of *Euglena gracilis* and *Astasia longa*. No electron micrographs of the latter species were obtained which show the chief features of the flagellum with the clarity of plate 38, *b*, nor were any found showing fraying of the flagellar core into fibrils. (Because of the greater sensitivity of *Astasia* to centrifugation, it was not possible to wash the culture enough to remove most traces of the nutrient culture solution; hence deposition of salts on the supporting film causes the appearance of a cloudy and granular background in most of the micrographs of flagellates dried from life.)

Plate 39, *d*, shows the internal differentiation of the flagellum into a denser central region which in some places appears to consist of two equal strands, a lighter surrounding region, and a sharp limiting membrane. The diameter of the whole is about 0.55$\mu$. In plate 39, *e* and *f*, and plate 40, *a*, the dense central core is apparent, but its double nature is not indicated, and the lighter surrounding substance is not sharply bounded, a limiting membrane being absent. Mastigonemes are seen on all three flagella. In plate 40, *a*, where these radiate out from the coiled flagellum, some are at least 3.6$\mu$ in length. In plate 39, *f*, there is no demarcation between the substance sheathing the flagellar core

and the bases of the mastigonemes, which are either so densely packed as to be individually indistinguishable or are partially coalesced with each other or with the sheath. The sheath on the side of the flagellum which does not bear mastigonemes is much thicker than previously seen. An apparent swelling of the sheathing substance is more evident in plate 40, *b*, where no mastigonemes are seen, and the sheath is fairly sharply demarcated, although without a limiting membrane. Diameter of the whole flagellum here is nearly 1.0$\mu$, while that of the undivided dense core is about 0.3$\mu$, approximately the same as in plates 39, *d* to *f*, and 40, *a*.

Plate 40, *c*, is of *Astasia longa* fixed in osmic-acid-formalin before drying and corresponds closely to plate 38, *g*, of *Euglena* similarly treated, except that the sheath in plate 40, *c*, is completely lacking, and the double nature of the dense core is very clear. Near the body of the organism the core decreases in diameter and becomes solid and darker. Plate 40, *d*, shows a flagellum fixed in Schaudinn's fluid before drying. Here the two strands of the flagellar core are distinct and may be seen crossing over one another. The notched margin of the core is barely evident. Mastigonemes are visible in some areas, but it is not clear whether the position of these is related to the crossing of the central strands.

*Rhabdomonas incurvum* (= *Menoidium incurvum*)

Light microscope preparations of *Rhabdomonas* flagella offered only two points of interest. One was the occasional fraying of the flagellum into what appear to be about 8 to 10 fibrils (pl. 40, *e*); the unfrayed portion of the flagellum is darkly stained by Loeffler's technique and without any evidence of a sheath. The other is the fact that, although no mastigonemes were seen on the flagella of *Rhabdomonas* in any stained preparations or in dark-field, stained flagella of *Monas vestita* lying close to or even superimposed on those of *Rhabdomonas* showed typical bilaterally arranged mastigonemes.

None of my electron micrographs of *Rhabdomonas* flagella shows any clear evidence of the fine longitudinal fibers seen in plate 40, *e*. Plate 40, *f*, reveals a small section of a *Rhabdomonas* flagellum, emerging from the cell at the left, in which the only apparent structures are two major fibers which cross over in the center of the picture. Plate 41, *c*, shows the tip (or perhaps broken end) of a flagellum, with some suggestion of internal longitudinal fibers, and with numerous (probably twenty or more) very fine fibrils fraying out at the end, but these appear to be continuous rather with the sheath than with the core of the flagellum. In the majority of the micrographs the axis appears as an undifferentiated shaft, with some evidence of beading, e.g., in plate 41, *a*. In plate 41, *c* and *d*, the axis is surrounded by a region of low density which is bounded by a limiting membrane, seen most clearly in plate 41, *d*.

Mastigonemes are clearly represented on most of the flagella; usually they appear along one side only of the flagellar axis, but in certain regions of plate 41, *a*, and plate 42, *b* and *c*, they are present for short distances on both sides. All figures on plate 42 show fibrillar structures in the region surrounding the axis, the fibrils in some areas passing transversely, possibly in a spiral or

circular manner. Plate 42, *a*, is of particular interest. The sheath at the upper extremity of the flagellum is disrupted, presenting a frothy appearance slightly suggestive of that in parts of plate 38, *c* and *d*, of *Euglena gracilis*. Farther down, the fibrils of the sheath would appear to be unwrapped, their free ends extending laterally as typical mastigonemes (see fig. 2). In plate 42, *b* and *c*, the continuation of transverse fibrils in the sheath as mastigonemes is very strongly suggested. Since it is obvious that the number of mastigonemes per unit length is greater than the number of transverse fibrils visible in the sheath, one may conclude that the latter fibrils represent bundles of subfibrils the frayed and extended ends of which are mastigonemes.

*Peranema trichophorum*

Results from all methods of study of this species corroborate Korschikow's (1923) report that the flagella consist of three main longitudinal fibers. Partially frayed flagella in Loeffler preparations are shown in plate 43, *a* and *b*. Although Korschikow's summary stated that the axoneme of the flagellum frayed into these three strands, implying the presence of a surrounding sheath, none of my preparations have clearly indicated the existence of a sheathing or embedding substance.

Mastigonemes have not been seen in any preparations on the flagella of *Peranema trichophorum*. In most Loeffler preparations, bacterial flagella are clearly stained, and slides made of cultures including both *P. trichophorum* and *Monas vestita* clearly demonstrate the pantonematic flagellum of the latter, but there are no indications of appendages on the flagella of *P. trichophorum*.

When living cells are treated with dilute gentian violet as recommended by Korschikow, the attached flagellum usually becomes free, both flagella become immotile, and both may separate into three clear, continuous, longitudinal fibers as described by him. When the concentration of the stain is low, the flagella usually remain unstained, or practically so, and the three fibers appear identical. After heavier staining, however, the dye is absorbed in the flagellum by only one of the three strands. In an unfrayed flagellum, a single straight axial fiber is stained, and colorless bands are seen symmetrically bordering it. In frayed flagella, the stained fiber is, as a rule, fairly straight, the unstained ones more or less twisted about it; the three seem equal in diameter. There is no indication of a sheathing substance.

After an exposure of some minutes to dark-field illumination, the flagella of *P. trichophorum* are usually shed, and soon begin to separate into three apparently equivalent strands. The originally smooth, optically homogeneous flagellum may start to fray at any point. The separation into strands may be complete, with all strands remaining intact for the entire length of the flagellum; or the fibers may fragment in the process of separation, the fragments usually coiling to form loops about or near the remainder of the flagellum (pl. 43, *c*).

Neither light- nor dark-field studies of living or fixed flagella gave any support

to the commonly offered description of the *Peranema* flagellum as tapering, or terminating in a free axial thread (e.g., Calkins, 1933). In all my preparations the flagellum appeared to be uniform in diameter and to end bluntly.

For the most part electron micrographs showed the flagellum of *P. trichophorum* as a fairly homogeneous opaque structure, about 1.1$\mu$ in diameter. In plate 44, *c*, there is indication of a subdivision into three heavy fibers which do not appear to be twisted about one another. Plate 44, *a* and *b*, which are micrographs of neighboring portions of a single flagellum, clearly show the fraying out of the three fibers. There is a suggestion of cross striation of single fibers in both figures.

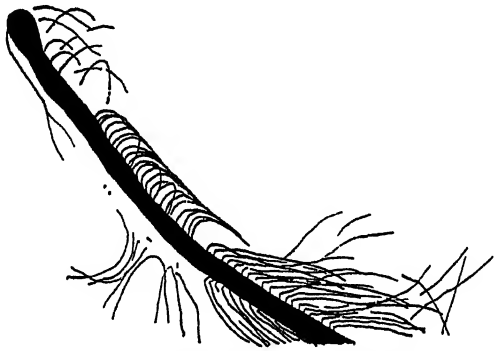

Fig. 2. Part of flagellum of *Rhabdomonas incurvum*, drawn from electron micrograph reproduced as plate 42, *a*. See text for discussion.

*Peranema acus* (= *Heteronema acus*)

Loeffler preparations of this species serve only to show a dark-stained axoneme and lighter sheath, with occasional indications of longitudinal fibrils within the axoneme. Nowhere is there any indication of the clear and consistent separation of the entire flagellum into longitudinal fibers seen in *P. trichophorum*. The addition of gentian violet to living organisms immobilizes the flagella and may stain the axoneme but causes no fraying. No mastigonemes or terminal appendages were seen in any stained preparation, although bacterial flagella were clearly demonstrated on the same slides.

In dark-field, normal active individuals have smooth, optically homogeneous flagella, which end bluntly without tapering; the shorter trailing flagellum appears slightly more slender than the long anterior one, which is about 0.7 or 0.8$\mu$ in diameter. After a few minutes in an illuminated field the flagella gradually disintegrate into granules or globules, leaving no trace of structure or organization. Disintegration may be preceded by bleb formation. Motility may be retained after the appearance of blebs and until surface disruption is quite marked.

Electron micrographs of the *Peranema acus* flagellum all show differentiation into a rather well-marked core and sheath. In plate 44, *f*, the core shows some fraying near the tip, where it is surrounded by the lighter sheath. The latter is quite smooth and well-defined; a number of very fine fibrils extend

from the distal end. Separation of the core into subfibers is also seen in plate 44, *d* and *e*, where the sheath appears swollen and irregular.

### *Entosiphon sulcatum*

In stained preparations the flagella of this species may show dark axonemes and light sheaths. No evidence was seen of fraying of any part of the flagellum. The two flagella show identical structure. Mastigonemes were never indicated, although the pantonematic flagellum of *Monas vestita* was demonstrated on the same slides. Fresh dilute gentian violet causes no fraying and reveals no differentiation other than the occasional appearance of close-set cross or spiral striations.

Dark-field examination shows the flagella of active organisms to be smooth, optically empty, and of a constant diameter of about 0.7$\mu$. Exposure to dark-field illumination causes them to slow down gradually, although some motility remains while blebs form, usually near or at the tips. Disintegration of the flagellum occurs explosively, portions of either flagellum suddenly transforming into granules and globules of varying sizes. The flagella usually are not cast.

One series of electron micrographs of *E. sulcatum* (pl. 45, *a* to *c*, *c*) shows in the center of the flagellum either two longitudinal strands or a cylinder of a fairly dense material; the center of this structure and the narrow region immediately surrounding it are less dense; this lighter region is bounded laterally by a sharp, thin, dark line. Peripheral to this is a layer of varying thickness, which appears to be fibrillar in structure. The fibrils seem to be packed in a fairly orderly manner and to pass transversely. Free ends are visible occasionally, but in no case seen are these as extended or numerous as are the mastigonemes of *Euglena*, *Astasia*, or *Rhabdomonas*. The body of the flagellum, exclusive of the fibrous layer, is about 0.5 to 0.7$\mu$ in diameter; the fibrous layer varies from almost nothing to a thickness nearly as great as the diameter of the flagellum. In some of the figures, swellings are seen in the light region immediately within the bounding membrane.

Another series of micrographs, exemplified by plate 45, *d*, shows a simpler differentiation of the flagellum into a uniformly dense, solid core, about 0.4 to 0.5$\mu$ in diameter, and a sheath of exceedingly low density and no visible structure, about 0.3$\mu$ in thickness.

### *Monas vestita*

A monad, *Monas vestita*, occurring as a contaminant in a strain of *Entosiphon sulcatum* which had been imperfectly isolated from a wild sample, served as a useful check on the adequacy of fixation and staining procedures, since a well-stained preparation always showed mastigonemes on the longer flagellum. No satisfactory electron micrographs were obtained of this species, the flagella of which are exceedingly susceptible to disintegration. When studied in dark-field, the flagella disintegrated rapidly, with no interval of quiescence which would permit detection of mastigonemes. However, specimens dried from life and stained according to Loeffler's method offer some evidence concerning the

question of bilateral or multilateral arrangement of mastigonemes on pantonematic flagella. As may be seen in plates 45, *f*, and 46, *a* and *b*, flagella of this species bear mastigonemes which appear on both sides of the axis, the filaments being shorter, probably finer, and arranged at shorter intervals along the flagellum than is the case in Loeffler preparations of *Euglena* and *Astasia*. If the mastigonemes take their origin from points all over the surface of the flagellum rather than in two discrete rows, one would expect, when the flattened, dried flagellum is seen in profile, to find individual mastigonemes of varying lengths, since only the distal portions would be visible of those filaments arising from the upper and lower surfaces of the flagellum. In the specimens I have studied this does not appear to be the case. All three figures reveal some regions along the flagella where the mastigonemes are not clumped or twisted and seem to be of remarkably uniform length; no shorter filaments can be detected. This is particularly evident in plate 46, *b*, where a portion of the row of mastigonemes has been torn away, presumably during or after fixation, from the surface of the flagellum; the uniformity of the mastigonemes is conspicuous (see fig. 3). Even in areas where clustering of the mastigonemes obscures their relative lengths, there is no indication of greater density near the body of the flagellum, as should be the case were the arrangement multilateral.

Fig. 3. Part of flagellum of *Monas vestita*, drawn from photomicrograph reproduced as plate 46, *b* See text for discussion.

*Sphaerella lacustris*

Electron micrographs reproduced in plate 46, *c* to *e*, show portions of the flagella of *Sphaerella lacustris*. A denser core, with some suggestion of fibrillar structure in plate 46, *c*, a lighter surrounding area, and an enveloping membrane are again apparent. Plate 46, *c* and *e*, show a wrinkling and buckling of the surface membrane, indicating a greater degree of shrinkage, or contraction, of the inner elements than of the membrane. Plate 46, *c*, reveals the distal end of one flagellum, with no suggestion of the terminal filament reported for both flagella of this species by Petersen (1929).

## DISCUSSION

It seems clear from all the descriptions given above that examination of stained flagella with the ordinary light microscope alone contributes little further to knowledge of the structure of this organelle. Such examinations obviously will continue to be useful in comparative studies, provided, as Owen (1947) points out, that a uniform method of preparation is employed, and that such a method includes a rapid and complete fixation of specimens before drying in order to avoid the extreme variability in appearance due presumably to partial disintegration of the flagellum before fixation. The results of my studies of stained specimens serve chiefly to corroborate certain evidence from dark-field and electron microscope work.

### *Euglena-Astasia-Rhabdomonas* Type

Of the six euglenoid species I have studied, the three for which more satisfactory series of micrographs were obtained will be discussed first.

All of my evidence leads to the conclusion that the flagella of *Euglena gracilis* and *Astasia longa* are similar in structure. Deflandre (1934) found such a resemblance in the flagella of *E. gracilis* and *A. dangeardii*, and Brown's (1945) report indicates that the flagella of *E. gracilis* and *A. klebsii* do not differ significantly. My brief examination of flagella of unidentified species of the two genera did not reveal any deviation from the common appearance. On the basis of my micrographs, it seems probable that the flagellum of *Rhabdomonas incurvum* is of a similar type.

My results agree with Brown's in showing a dense axial core which either is normally present as two discrete fibers or is readily separable into two (cf. Dellinger's [1909] report of a flagellum of *Euglena sp.* separable into four strands). These major fibers may in turn be divided into fibrils, of which a maximum of nine or ten per flagellum is probable. Where such fraying has occurred, there is little if any trace of the former association in two fibers. The diameter of the more slender fibrils in plate 38, *c*, is probably between 350 and 600Å. These figures for the number and diameter of fibrils show a striking conformity to those for fibrils in cilia, flagella and sperm tails examined with the electron microscope by Jakus and Hall (1946) and the earlier authors reviewed by Schmitt (1944). Foster *et al.* (1947) do not give the number or dimensions of fibrils in their electron micrographs of protozoan flagella, but their figure 10 indicates a larger number than previously seen. They also demonstrated an intimate twisting of the fibrils in their "fibrous" type of flagellum, a phenomenon not described by other investigators.

Evidence for the nature of the sheath is much less conclusive. The only structures other than the longitudinal fibrils described in the reports of Schmitt, and of Jakus and Hall, were in some cases an ill-defined cross striation and in others (mammalian sperm tails) a helically wound fibril surrounding the fibrillar core. Saxe (1947) described for *Octomitus intestinalis*, and Foster *et al.* showed for unidentified flagellates a sheath of lower density than the axial core, but with no apparent structure. Brown (1945) described for the flagella of *Euglena gracilis* and *Astasia klebsii* a sheath which contains, or consists of, what he interprets as a closely wrapped helical fibril. His evidence is drawn for the most part from micrographs which present two rather diverse types of appearance. On the one hand, his plates 2, A; 3, A and B; 5 and 7 show a sheath which (except for the upper part in figure 7) is clearly distinct from and less dense than the axial core; here the fibrils in the sheath extend well away from the axis and could be oriented circularly or as one or more helices of low pitch. On the other hand are plates 1, B; 2, B; and 9, where the sheath, if present at all, is closely appressed against the axis and there are visible nodules along the sides of the axoneme which could be interpreted as helical fibrils seen in profile, the spiral being of higher pitch than in some of the examples just described.

The majority of my electron micrographs of *Euglena gracilis* and *Astasia longa* show the sheath either as a variously swollen and almost structureless substance, as in plate 40, *b*, of *Astasia*, or as an area of almost complete electron permeability bounded by a sharp, thin, limiting membrane, as in plate 38, *b*, of *Euglena*. Only in plate 38, *c* and *d*, of *Euglena* is there any indication of a fibrillar sheath. In plate 38, *f* and *g* of fixed specimens, the serration of the margins of the axial fibers might correspond to the nodules interpreted by Brown as sections of a helical fiber, but in these cases a sheath is visible and structureless.

The micrographs of *Rhabdomonas* flagella clearly show fibrillar elements in the sheath. As pointed out above, plate 42, *a*, *b*, and *c*, strongly suggest that the mastigonemes actually are the somehow freed and frayed ends of fibrils which compose or contribute to the sheath. Certain evidence drawn from the work of previous authors lends some support to this observation. Foster *et al.* point out that in some of their electron micrographs, mastigonemes appear to arise in groups from nodules visible along the side of the shaft of the flagellum, which they believe correspond to the nodules interpreted by Brown as the helical fibril of the sheath. These nodules, however, in the micrographs of Foster *et al.*, were limited to one side of the flagellum. Brown's plate 11 of the pantonematic flagellum of *Ochromonas variabilis* shows mastigonemes apparently arising from the nodules, which here appear on both sides.

None of my micrographs indicates a helical configuration of the fibrils of the sheath as strongly as do those of Brown (see pl. 37, *a*). In my plate 42, *a* and *b*, and fig. 2, transverse or slightly oblique fibrils, occurring at fairly regular intervals, are most clearly seen in a region immediately adjacent to the dense axial core, and, as just stated, in some areas these appear to continue directly as clusters of mastigonemes. On the basis of these figures, it appears quite possible that the fibrils arise from the axial core, pass laterally through the region of the sheath (perhaps through a substance of low density which holds them in place and prevents fraying) and then fray out as mastigonemes. In some areas the fibrils, or a majority of them, are not so frayed, but seem to run a longitudinal or spiral course at or near the surface of the sheath. The close packing of fibrils occupying the region of the sheath in plate 41, *b*, might be explained by assuming that the sheath fibrils have separated into their component mastigonemes which, instead of extending laterally, adhere to the surface of the flagellum.

The very smooth surface of the flagellum as seen in dark-field preparations indicates that there is some sort of formed membrane surrounding the living flagellum, that is, that the sheath could not be composed of helical or transverse fibrils alone. This is borne out by the appearance of a sharp line bounding the flagellum in micrographs such as plates 38, *b*, and 39, *d*, as well as by observations such as Korschikow's (1923), cited above, on disintegrating flagella. The limiting membrane apparently is destroyed by most chemical fixatives and frequently by drying.

Several of Brown's micrographs and most of mine of *Euglena* and *Astasia*

offer evidence of a nonfibrous substance occupying the space between axoneme and limiting membrane which seems to be the most variable of all the elements of the flagellum. The region it should occupy is distorted where the flagellum comes in contact with foreign objects (pl. 38, *b* and *c*); it may appear swollen and structureless (pl. 40, *b*); it apparently is often destroyed during the processes of fixing and staining for the light microscope. Mainx (1928) observed in dying euglenoid flagella that this substance is probably fluid, or becomes so as disintegration sets in.

From these accumulated observations, it seems probable that flagella of the euglenoids under discussion consist of an "axoneme" composed of fibers comparable in number and diameter to those of other flagella, cilia, and sperm tails, but here arranged in two bundles, and a composite "sheath" which includes some fibrillar elements, a probably semifluid matrix, and a limiting membrane; the fibrils of the sheath, under some conditions at least, fray out laterally as mastigonemes.

Unfortunately, none of the micrographs thus far published has constituted a convincing demonstration of the internal structure, or external contours, of a flagellum in a lifelike state. The differences between micrographs of specimens which have been fixed before drying and those which have not are so great as to indicate that the fixatives used have modified the structure of the flagellum and its electron permeability to a significant degree. On the other hand, we have no proof that any of the flagella dried without previous fixation have not suffered considerable destruction during drying, and the variability in appearance among such flagella presents problems which cannot be solved until we are more certain of the organization of the normal flagellum.

The present work serves to point up certain problems which must be given attention in further research. Prominent among these is the question of the orientation of the fibril or fibrils in the sheath, i.e., whether they arise from the axial core and pass radially through the sheath, or follow a continuous spiral course at or near the surface of the sheath. The former interpretation would seem more logical on the basis of my micrographs of unfixed specimens, but the latter must be considered on the basis of Brown's work. In his plates, there is no discrete limiting membrane; the only fibrillar structure visible in the sheath is clearly transverse and probably helical. No free ends, with the exception of an occasional mastigoneme, are seen. Another significant puzzle in some of Brown's and many of my plates is the absence of any evidence of fibrils within the sheath, while mastigonemes, which almost certainly are derived from such fibrils, are visible.

The mastigonemes are frequently at least four or five times as long as the diameter of the flagellum, so that if they are frayed or unwound from a helical fiber in the sheath, each bundle of mastigonemes before fraying would have to extend over considerably more than one complete gyre of the helix. Associated with this is another enigma. Why do the mastigonemes in the euglenoid flagellum appear, in the vast majority of cases, on one side only of the flagellum? That this is not invariably so, in *Rhabdomonas* at least, is evidenced by several

of my micrographs, but certainly in the majority of electron microscope preparations and in all light microscope preparations I have seen, the mastigonemes are unilateral in euglenoids, but not in other groups. The facts that they rarely do occur on both sides and that when they are unilateral they are usually seen on the outer side of any bend in the flagellum might suggest that they are merely swept to one side by the last movement of the flagellum before fixation. But if this is the case, why does the same phenomenon not occur more frequently in other species? My plate 42, *b*, would certainly indicate that the origin of the fibrils is not limited to one side of the axoneme.

Another fact that needs explanation is the marked regularity of mastigonemes as seen with the light microscope, especially in nigrosin preparations and in dark-field. Since the mastigonemes leave the surface of the flagellum in clumps, the fraying of which is visible in electron micrographs, it might be suggested that each clump constitutes one "mastigoneme" seen with the light microscope. But even this could not account for the difference in number and distribution of mastigonemes seen with the two instruments. The distance between clumps of mastigonemes at the sheath of the *Rhabdomonas* flagellum averages about 0.1 to 0.15$\mu$; measurements taken from Brown's plates 2, A and 3, A (printed here as pl. 37, *a*) of *E. gracilis* indicate that the distance between gyres of the helix is 0.07 to 0.15$\mu$. But in nigrosin and dark-field preparations of *Euglena* and *Astasia*, mastigonemes arise at intervals of about 0.9$\mu$ along the flagellum. According to Deflandre (1934) the interval was 1.0 to 1.5$\mu$. It becomes necessary to assume that several clumps of mastigonemes must be twisted together, in some quite regular fashion, to form one supermastigoneme demonstrable with the light microscope.

This is reminiscent of the case of bacterial flagella, where dark-field and electron microscope examinations show a much larger number of finer fibrils than are visible in stained preparations. Pijper (1938, 1941, 1946) saw the flagella of dying typhoid bacilli under dark-field illumination break up first reversibly into two flagella and then irreversibly into numerous very fine filaments. Johnson, Zworykin and Warren (1943) reported from electron microscope studies that larger fibrils of the flagella of *Achromobacter harveyi* were about 0.01$\mu$ in diameter and were probably made up of subfibrils averaging 0.016$\mu$; Knaysi (1912) estimated that the width of the individual flagellar fibril of *Aerobacter cloacae* would be about 0.03$\mu$. The diameter of mastigonemes seen in my electron micrographs probably does not exceed 0.025$\mu$, or a size comparable to the finest units of the bacterial flagellum. Of interest in this connection is the recent opinion of Pijper (1946, 1947, 1948) that bacterial flagella are simply polysaccharide chains spun out from the slime layer coating the cell, which may twist together to varying degrees depending on the activity of the organism and other factors. Pijper's position has been attacked by Conn and Elrod (1947) and by Van Iterson (1947).

It should be possible, with a completely optimal dark-field system, to detect fibrils perhaps even as fine as the mastigonemes seen on electron micrographs. At any rate, further dark-field examination should yield much valuable infor-

mation regarding conditions governing the appearance of mastigonemes. I have already demonstrated that under optical conditions as nearly as possible identical, mastigonemes sometimes appear and sometimes do not. This might be due to an imperfection of the optical setup with the result that where the mastigonemes failed to clump, the finer fibrils were invisible. (Reichert [1909] reported that many bacterial flagella were not visible in dark-field in distilled water or non-electrolyte-containing media, presumably due to phenomena of adsorption or to a failure of the flagellar fibrils to clump into visible units.) Or it might be due to the actual absence of mastigonemes under certain conditions. As has been seen, mastigonemes are sometimes lacking in electron micrographs of *Euglena*, *Astasia*, and *Rhabdomonas* flagella, as well as in all other types of preparations studied. They might, of course, be destroyed in the process of fixation.

However, unless mastigonemes can be demonstrated on healthy living flagella (both Vlk and I saw them on motile but dying organisms), it remains possible that they are products of disintegrative protoplasmic changes. Recent researches do not seem to support the suggestions of earlier authors that mastigonemes are independently motile (Fischer, 1894, and others), or rigid and serving to increase the effective surface of the flagellum (Mainx, 1928). I cannot understand the brief suggestion of Foster *et al.* (1947) that the mastigonemes could contribute as such to movement of the flagellum. Furthermore, to assume that a great brush of passive, flexible, long filaments such as seen in electron micrographs, or even of stouter clumps of these seen in dark-field, exists normally on such rapidly moving flagella as those of the species studied seems to me difficult. In view of their integrity in a liquid medium in dark-field preparations and their clearly fibrillar structure as indicated in my micrographs, I believe that the possibility of their being liquid substance extruded through pores in the sheath and subsequently coagulated, as tentatively suggested by Brown (1945) and maintained by Owen (1947), is unlikely.

If it is true that mastigonemes appear only after some change from the normal organization of the flagellum, the fact remains that they most definitely are not artifacts, but distinct structures bearing some relationship to that normal organization.

There seems to be a tendency for some fibrous proteins to group themselves as fibers with a diameter of something under 250Å. Hall, Jakus, and Schmitt (1946) published electron micrographs of myosin filaments from muscle extracts which the authors stated to be 50 to 250Å wide. The filaments in intact muscle fibrils were indefinitely long but they fragmented during extraction to variable lengths, depending on the species of animal, but not usually exceeding 1.5$\mu$. An electron microscope study by Wolpers (1941) revealed the protein portion of erythrocyte membranes as a fibrous network, the fibers having a diameter of about 150Å. Fibrils of smaller or larger diameter have been seen in preparations of fibrin, collagen, and nerve axoplasm (Schmitt, 1944; De-Robertis and Schmitt, 1948). The diameter of the mastigoneme is less than 250Å. While it is possible that this tendency of fibrous proteins to occur in

bundles of this order of size may have some bearing on the formation of mastigonemes, a further explanation is necessary for their characteristic distribution and orientation. Since it appears likely that the mastigonemes are directly related to fibrous structures in the sheath, such an explanation will depend on a demonstration of the nature and position of these fibrous structures in life.

Thus far, all flagella and similar organelles which have been extensively investigated have revealed a core made up of longitudinal fibrils. Schmitt (1944) has suggested that "the submicroscopic longitudinal fibrils . . . provide a unique capillary system in which a change in distribution of interfibrillary water might cause the undulatory contortions characteristic of the sperm tail and the pendular beating of cilia and flagella" (p. 38). Actually, undulatory contortions are characteristic of many flagella as well as of sperm tails; it may be that the division of the longitudinal core into two bundles revealed in the euglenoids might have some relationship to this type of movement. The helical fibril of the sheath, which has been demonstrated or indicated only for sperm tails and flagella bearing mastigonemes, could conceivably function in an undulating type of movement, particularly if the helix is made up of numerous finer mastigonemes.

## Other Types

Of the remaining three euglenoid flagella studied, the only one which thus far offers fair evidence of a structural similarity to the *Euglena-Astasia-Rhabdomonas* type is that of *Entosiphon sulcatum*. The heavy double core, surrounding light area, and sharp limiting membrane have been pointed out, and are strongly reminiscent of plate 38, *b*, of *Euglena gracilis*. However, the abundance of fibrillar material external to all of these (pl. 45, *a* to *c*), exhibiting a noticeable transverse organization, is unparalleled in any micrographs of the above species. I see no reason to doubt that these fibrils are related to the mastigonemes of *Euglena*, but their occurrence in an apparently organized form outside of the membrane which in the preceding species seems to represent the surface of the flagellum, is most puzzling. That this organization is not rigid is indicated by plate 45, *c*; the smooth surface of the flagellum seen in dark-field illumination must also be considered.

Regarding the flagellum of *Peranema acus*, the significant facts which emerge from my study are that, as might be expected, the core is fibrillar, the number of fibrils being as yet undetermined; and that a sheath is present, whose constituents have still to be exposed. For the flagellum of *Peranema trichophorum*, a comparable paucity of evidence prevents any conclusion other than the already recognized fact of its tripartite nature. The difference in the reaction of the three fibers to gentian violet staining is of interest. It is probably safe to assume that further study will show a division of the three major fibers of the *P. trichophorum* flagellum into fibrils, and that some sort of sheath will be found. In view of its extraordinarily large size (around 1$\mu$ in diameter as contrasted with about 0.5$\mu$ in *Euglena gracilis*, for instance) the peculiar structure of this flagellum is not surprising, and more information concerning its submicroscopic organization is highly desirable.

My light microscope studies of the flagellum of *Monas vestita* serve chiefly to emphasize the variability of conditions governing the appearance of mastigonemes on flagella of different species. As has been pointed out, mastigonemes were clearly seen on flagella of *Monas* in Loeffler preparations which failed to reveal them on the flagella of *Rhabdomonas*, *P. trichophorum* and *Entosiphon*.

The absence of any terminal appendage on the flagellum of *Sphaerella lacustris* in plate 46, *c*, is of interest inasmuch as this species was reported by Petersen (1929) to have two acronematic flagella. He stated that specimens fixed in osmic acid vapor before drying failed to show the terminal filament while those dried from life frequently showed it clearly. The fact that in plate 46, *c*, the sheath surrounds the tip of the fibrous core may indicate that when a terminal filament occurs in this species it is an extension of the sheath rather than of the axoneme.

As noted above, I have occasionally seen in dark-field preparations, particularly on the flagellum of *Astasia*, a tenuous wavy thread spun out from the tip of the dying flagellum. Similar structures appeared in nigrosin mounts. Ellison (1945), studying zoospores of fungi with the light microscope, reported in several instances acronematic flagella, blunt flagella and (or) knobbed flagella present on varying proportions of living as well as stained individuals examined within a single species; in acronematic flagella, base and filament varied inversely in length. He suggested that the knobbed end resulted from an unexplained more fluid condition of the substance which otherwise might be drawn out as a terminal thread. Such evidence indicates that the terminal filament reported for so many flagella (see table 1) may in some cases be a transient, though naturally occurring structure, and in others a product of disintegration. In still others it seems to be a consistently formed appendage (e.g., *Pentatrichomonas hominis*, Kirby, 1945). As far as I know, no electron micrographs showing acronematic flagella have been published.

While the universality of longitudinal fibrillar organization in the core of all types of flagella and cilia is becoming increasingly apparent, and evidences for the occurrence of fibrillar structures in the sheath are multiplying, the significance of the nonfibrous substance of the sheath must not be overlooked. The individuality of the flagella of various species is attested by their peculiar patterns of movement, responses to the rigors of dark-field illumination, and staining reactions. It seems probable that the sheath is responsible for many of these specific characteristics.

## Taxonomic Implications

The use of flagellum type (i.e., presence and kind of appendage on the flagellum) as a taxonomic character, indicative of phylogenetic relations, has been suggested by Petersen (1929), Vlk (1938), Brown (1945), and Copeland (1947), among others. The data accumulated in table 1, copied from Vlk, strongly favor the conclusion that, at least where flagellar appendages are readily demonstrable, their appearance is strikingly uniform within the larger systematic categories. However, the character must be employed with caution.

Clearly the absence of any appendage cannot be assumed to be diagnostic, as proven by the failure of Mainx (1928), Petersen (1929), Deflandre (1934) and the present writer to demonstrate mastigonemes on the flagella of some of the euglenoid species studied in each case; by Ellison's (1945) discovery of simple, acronematic or knobbed flagella on individual zoospores of a single fungus species; and by the absence of a terminal filament in my preparations of the supposedly acronematic flagellum of the phytomonad, *Sphaerella lacustris*. Furthermore, until we have a much better understanding of the nature of mastigonemes, their relation to the sheath, and the factors governing their occurrence, as well as of the submicroscopic structure of acronematic flagella, it remains possible that mastigonemes may be demonstrated under some conditions on the flagella of species in groups such as the phytomonads.

However, these facts do not preclude the use of positive flagellar characters for systematic and phylogenetic studies. Vlk has pointed out two cases of organisms formerly placed in the Chrysomonadina in which evidence of flagellar types supported evidence from other sources indicating that their taxonomic position should be changed.

The stichonematic flagellum seems to be a clearly identifiable type and so far has been demonstrated convincingly only on some euglenoids. Moreover, all euglenoid species reported to have such flagella belong to the conventional families Euglenidae and Astasiidae (see table 1), with the exception of *Urceolus cyclostomus*, seen by Vlk (1938). His report of a stichonematic flagellum for this species is based on examination of a single organism and cannot be accepted without corroboration. The flagella of *Entosiphon sulcatum*, as described above, do not show typical mastigonemes, but seem to have a fibrillar organization somewhat modified from the *Euglena* type. It may be quite significant if the distinction evident so far is found to be consistent, that is, if all organisms usually placed in the Euglenidae and Astasiidae should be found to have more or less typical stichonematic flagella, while members of the Peranemidae show different flagellar organization.

Hollande (1942) and Pringsheim (1948) have discussed groups of pigmented and colorless species (normally separated in two families) which seem to be more closely related to each other than to other green or unpigmented forms respectively. The very close similarity in structure and reaction of the flagella of species *Astasia* and *Euglena* given close comparative study so far, is interesting in this respect, since the three *Astasia* species, *A. longa*, *A. dangeardii* and *A. klebsii*, are all rather similar in other details, and since *A. longa* differs from *E. gracilis*, the species of *Euglena* studied in each case, only in the lack of chromatophores and eyespot (Pringsheim, 1948). It seems possible that careful examination of flagellar structure might aid in clarifying relationships among green and colorless forms and perhaps even help to establish some more natural classification scheme than the present very unsatisfactory separation of Euglenidae and Astasiidae (see Hollande, 1942).

Schiller (1925) placed two green marine genera with stigmas but without reservoirs in the order Euglenida as primitive forms. It should be interesting

to study the flagella of these organisms; demonstration of unilateral mastigonemes would constitute strong support of Schiller's position. If his species are indeed primitive euglenoids, their flagellar type would be significant in any event.

Contrasted with the rather similar flagellar organization found in all other euglenoids studied are the widely divergent types of flagella seen in members of the family Peranemidae. Further study may, of course, reveal a common basic pattern, yet the flagella of *Peranema trichophorum* and *P. acus* remain strikingly different. In spite of this difference, the resemblance between the two organisms in every other respect is so close that I see no adequate reason for altering their congeneric status (Pitelka, 1945).

Deflandre (1934) described a stichonematic flagellum for the dinoflagellate, *Glenodinium uliginosum*. He admitted that his evidence was not perfectly convincing, and when Vlk (1938) repeated the work and examined other dinoflagellates, he came to the conclusion of Entz (1928) that at least the transverse flagellum was band-shaped, the longitudinal one sometimes being acronematic. Hence I believe that Copeland (1947) is in error in drawing on Deflandre's evidence to support a close relationship between the dinoflagellates and euglenoids.

## SUMMARY

The flagella of six species of the order Euglenida, *Euglena gracilis*, *Astasia longa*, *Rhabdomonas incurvum*, *Peranema trichophorum*, *Peranema acus* and *Entosiphon sulcatum;* one chrysomonad, *Monas vestita;* and one phytomonad, *Sphaerella lacustris*, have been studied, using stained specimens for light microscope examination, living organisms seen in dark-field, and electron micrographs of dried specimens.

The flagella of *E. gracilis*, *A. longa*, and *R. incurvum* were found to consist of an axoneme, composed of about nine longitudinal fibrils 350 to 600Å in diameter, arranged in two compact, parallel bundles; and a sheath which includes fibrillar elements, a probably semi-fluid matrix, and a limiting membrane. Under some conditions, apparently always associated with death of the organism, the fibrils of the sheath fray out along one side only (rarely more) of the flagellum into fine lateral filaments, the mastigonemes, which in electron micrographs appear very numerous, without any regularity of arrangement, and of a diameter estimated at less than 250Å. Mastigonemes were seen on motile but dying organisms in dark-field, where they appear much less numerous, thicker, and very orderly in orientation. The number and diameter of longitudinal fibrils in the axoneme correspond closely to those in all other flagella, cilia and sperm tails extensively examined with the electron microscope, and adds to the probability that the axoneme is the contractile part of the flagellum.

The flagella of *Entosiphon sulcatum* show a basic similarity to the *Euglena* type, but the fibrillar structure of the sheath is different. *Peranema acus* has an axoneme which is probably fibrillar; no structure has been observed in the sheath. The entire flagella of *P. trichophorum* separate rather readily into

three longitudinal fibers; no internal differentiation has been detected. None of these species bears mastigonemes.

The flagella of *Sphaerella lacustris*, which, in common with those of other phytomonads, are generally supposed to bear single terminal fine filaments, are seen to end bluntly, with no decrease in diameter, the probably fibrous axoneme being completely surrounded at its tip by the sheath. No electron micrographs have been obtained of the flagella of *Monas vestita*, but stained specimens almost invariably show mastigonemes on both sides of the longer flagellum.

Unilateral mastigonemes have been convincingly demonstrated only on members of the euglenoid families Euglenidae and Astasiidae, and their occurrence may be characteristic of this group, whose present familial division is unnatural; the Peranemidae, however, apparently lack them.

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# PLATES

## PLATE 37

*Euglena gracilis*

*a*. Electron micrograph of one flagellum, showing fibrillar structure in sheath. End of flagellum adheres to torn strip of supporting membrane, which is curved back on itself at lower right. Fixed in osmic acid before drying. Micrograph lent by Dr. Harley P. Brown.

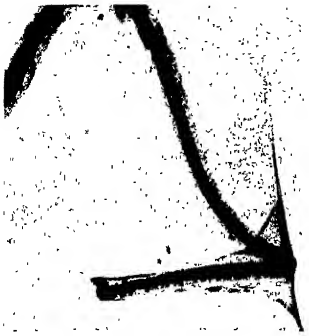
*b*. Photomicrograph of cast flagellum, partially coiled, frayed at upper left. Dried from life; Loeffler stain. × 1700.

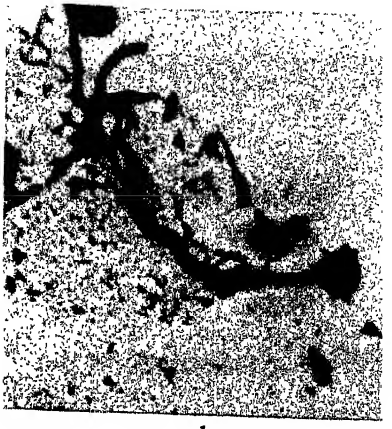
*c*. Photomicrograph of cast flagellum, showing mastigonemes. Dried from life; Loeffler stain. × 1700.

*d*. Photomicrograph of flagella of two individuals, showing mastigonemes. Dried from life; Loeffler stain. × 1700.

*e*. Photomicrograph of two cast flagella, showing exceptionally large mastigonemes. Dried from life; nigrosin stain. × 1700.

*f*. Drawing of cast flagellum, with characteristic bleb at proximal end, and mastigonemes, as seen in dark-field. Drawn from camera lucida sketch. × 1800 app.





*b*

*d*

Electron micrographs of *Euglena gracilis* all × 8100 approx

*a* Portions of flagella of two individuals. One piece to right lies close to, and is partially obscured by, body of cell. Dried from life.

*b* Portions of flagella of two individuals. Part of cell body appears at bottom of figure; both flagella lie partly under and hidden by, this cell. Heavy dark line in right-hand micrograph caused by two flagella crossing and lying partly superimposed for about 1½ inches; longer flagellum continues straight to upper left, shorter curves back toward cell. Dried from life.

*c* Portion of flagellum showing fraying. Dried from life.

*d* Two segments of flagella partially frayed. Dried from life.

*e* Small segment of flagellum. Dried from life.

*f* Two portions of flagella, lying partly superimposed; part of cell body visible at bottom of figure. Fixed in osmic-acid-formalin before drying.

*g* Part of flagellum; mastigonemes barely visible at upper left (see fig. 1). Fixed in osmic-acid-formalin before drying.

a
b
c
e
f
d
g

## PLATE 39

*Astasia longa*

*a* Photomicrograph of cast flagellum coiled in characteristic manner Dried from life, Loeffler stain × 1700

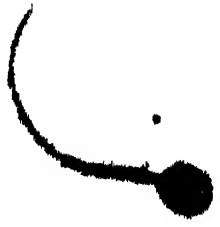
*b* Photomicrograph of two cast flagella, the upper one frayed Dried from life, Loeffler stain × 1700

*c* Drawing of cast flagellum showing blob near one end, and mastigonemes, as seen in dark-field × 2500 app

*d* Electron micrograph of portion of flagellum Dried from life × 8000 app

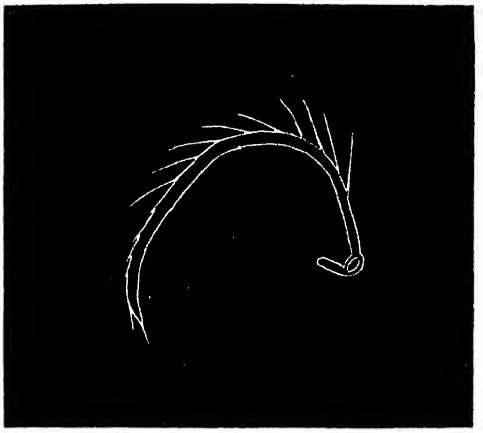
*e* Electron micrograph of part of a flagellum, mastigonemes along right margin, appearance of fibrils along left margin suggests mastigonemes, but probably is artifact Cell body at lower left Dried from life × 8000 app

*f* Electron micrograph of part of flagellum Dried from life × 8000 app



a

b



c

d

PLATE 40

*a*. Electron micrograph of coiled, cast flagellum of *Astasia longa*. Dried from life. × 8000 app.

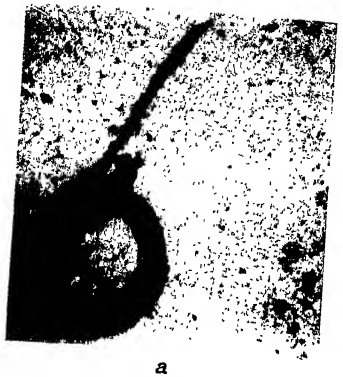
*b*. Electron micrograph of portion of flagellum of *A. longa*. Dried from life. × 8000 app.

*c*. Electron micrograph of part of flagellum of *A. longa*, emerging from cell at lower left. Fixed in osmic-acid-formalin before drying. × 8400 app.

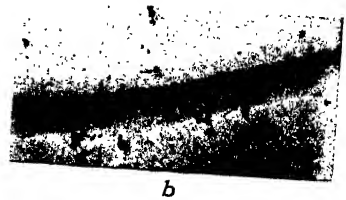
*d*. Electron micrograph of flagellum of *A. longa* emerging from cell at bottom; mastigonemes barely visible at upper left. Part of empty periplast at lower left, showing characteristic striations. Fixed in Schaudinn's fluid before drying. × 8400 app.

*e*. Photomicrograph of cast flagellum of *Rhabdomonas incurvum*, partly frayed. Dried from life; Loeffler stain. × 1700.

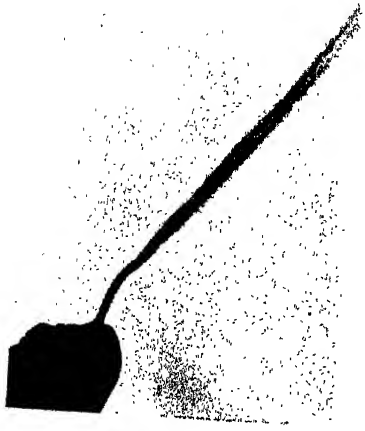
*f*. Electron micrograph of part of flagellum of *R. incurvum*, probably emerging from cell at left. Dried from life. × 8000 app.



a



b



c

d

e

f

## PLATE 41

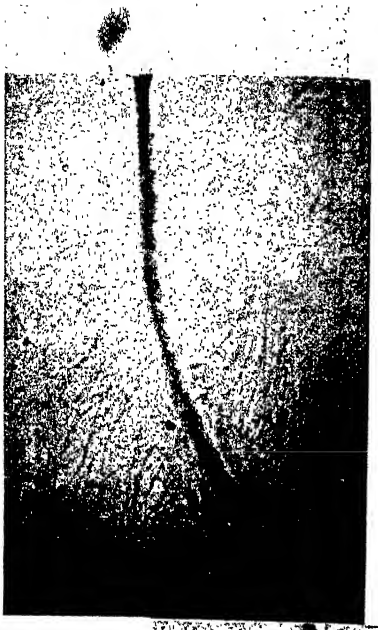
Electron micrographs of *Rhabdomonas incurvum*, all × 7500 app.

*a*. Entire flagellum, tip perhaps broken. Dried from life.

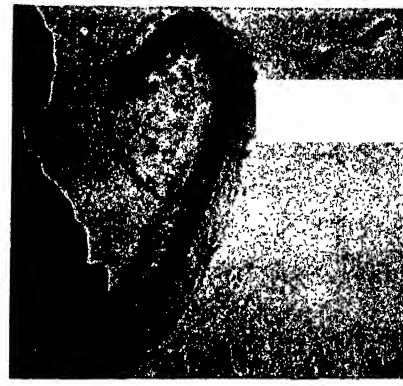
*b*. Portion of flagellum. Dried from life.

*c*. Tip of flagellum. Dried from life.

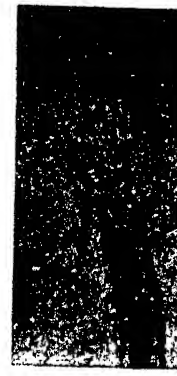
*d*. Tip of flagellum. Dried from life.



a



b



c

d

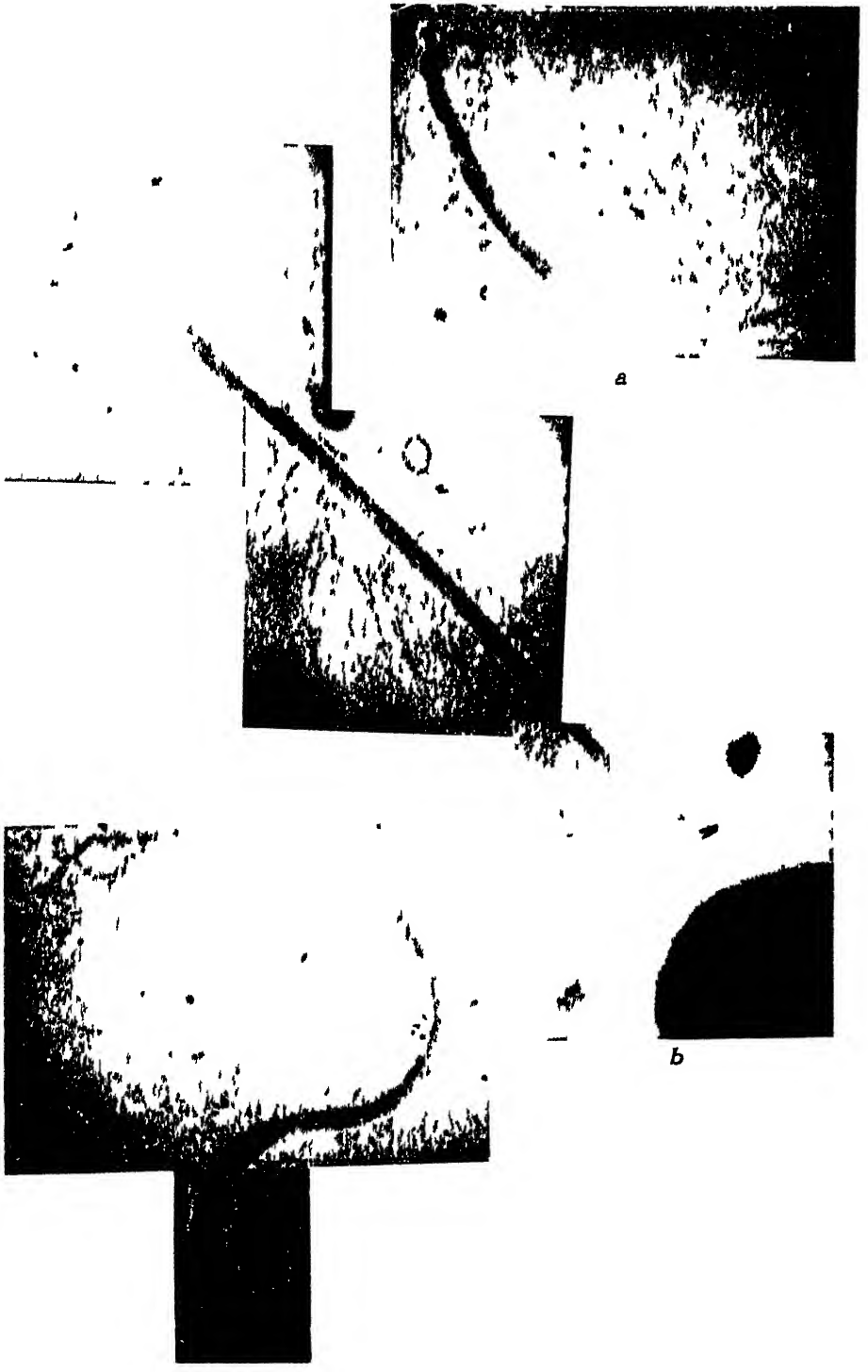
## PLATE 42

Electron micrographs of *Rhabdomonas incurvum*, all × 7500 app.

*a* Portion of flagellum Dried from life (see fig 2)

*b* Entire flagellum A cell body and part of at least one more flagellum at lower left Dried from life

*c* Cast flagellum Dried from life


a
b

## PLATE 43

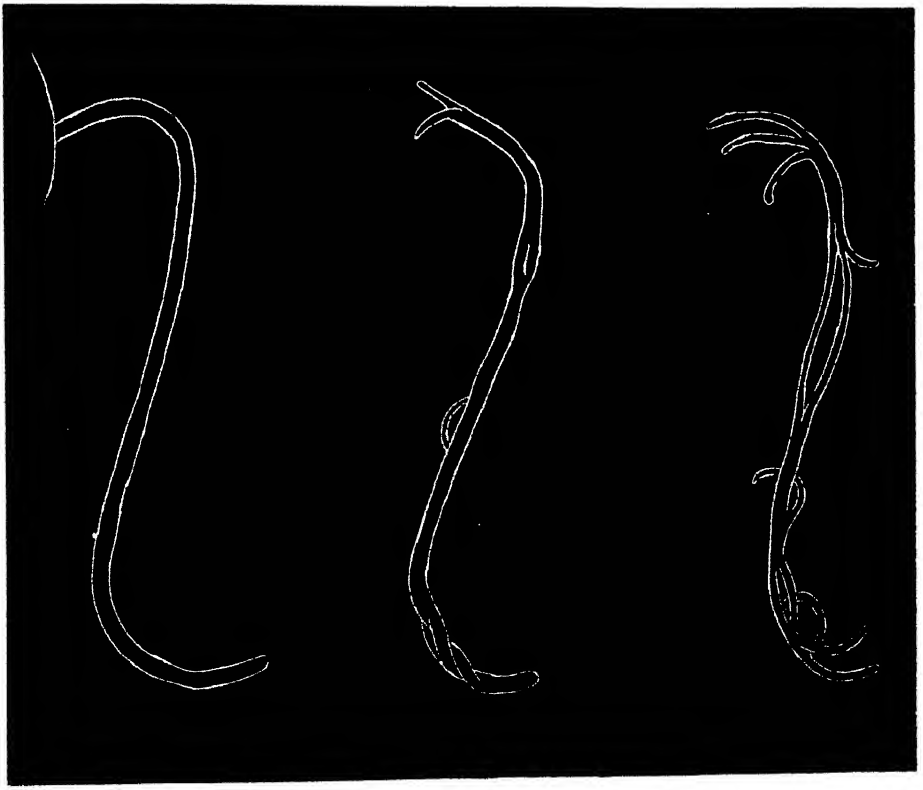
*Peranema trichophorum*

*a*. Photomicrograph of flagellum emerging from cell at lower left, separated proximally into three fibers. Dried from life; Loeffler stain. × 1700.

*b*. Photomicrograph of parts of two flagella, probably both from cell at left, both partially frayed. Dried from life; Loeffler's stain. × 1700.

*c*. Drawings of one flagellum of *P. trichophorum* in dark-field, sketched at successive intervals of a few seconds to show progressive splitting into three strands. × 1500 app.

a
b



## PLATE 44

*a* and *b*. Electron micrographs of neighboring regions of single flagellum of *P. trichophorum*. Fiber curving to right in lower figure probably loops back to cross remaining two strands. Some cross-striation of individual fibers indicated. Dried from life. × 8000 app.

*c*. Electron micrograph of base of one flagellum of *P. trichophorum*, emerging from cell. Dried from life. × 8000 app.

*d*. Electron micrograph of portion of flagellum of *Peranema acus*. Dried from life. × 8000 app.

*e*. Electron micrograph of portion of flagellum of *P. acus*, probably broken end. Dried from life. × 8000 app.

*f*. Electron micrograph of end of flagellum of *P. acus*. Dried from life. × 6500 app.

a

b

f

## PLATE 45

*a* Electron micrograph of portion of flagellum of *Entosiphon sulcatum* Dried from life × 8400 app

*b* Electron micrograph of parts of two flagella of *E. sulcatum*, bacterial cells and debris at right Dried from life × 8400 app

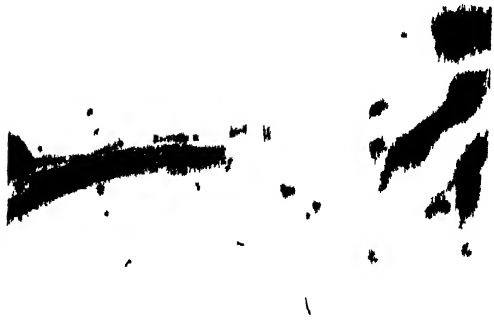
*c* Electron micrograph of part of flagellum of *E. sulcatum*, bacterial cells lying in contact with lower margin Dried from life × 8400 app

*d* Electron micrograph of parts of two flagella of *E. sulcatum* Part of cell of this species at lower right Pointed objects at upper right are mucus rods released by *Monas vestita* Dried from life × 8000 app

*e* Electron micrograph of short segment of flagellum of *E. sulcatum* Dried from life × 8400 app

*f* Photomicrograph of flagellum of *Monas vestita*, cell at lower right Dried from life Loeffler stain × 1700

a

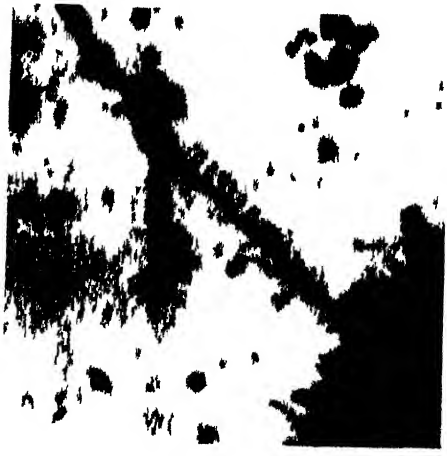


b

c

d

e



f

## PLATE 46

*a*. Photomicrograph of two individuals of *Monas vestita* with flagella. Mucus rods seen radiating from cells. Dried from life; Loeffler stain. × 1700.

*b*. Photomicrograph of part of flagellum of *M. vestita*, emerging from cell at lower right. Part of row of mastigonemes torn away at upper center (see fig. 3). Dried from life; Loeffler stain. × 1700.

*c*. Electron micrograph of end of flagellum of *Sphaerella lacustris*. Dried from life. × 8000 app.

*d*. Electron micrograph of bases of two flagella of *S. lacustris*. Dried from life. × 8000 app.

*e*. Electron micrograph of part of flagellum of *S. lacustris*. Supporting film is torn and folded. Dried from life. × 8000 app.

*b*

*d*